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A GENERAL METHOD FOR INVESTIGATING THE EQUILIBRIUM OF GENE FREQUENCY IN A POPULATION*

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IT is a well known fact that nearly all, if not all, natural populations are in some degree polymorphic. On a phenotypic level, this simply means that there exist a number of different morphological or physiological types in the population. On a genotypic level, this often, although not always, means that there exists in the population more than one allelomorph at a given locus. The fact that alternate states of a gene persist over long periods in a population has been one of great interest to students of evolution.

In a recent paper LI (1955) discussed various cases of equilibrium and demonstrated that the adaptive value or average fitness of a population is maximized at the equilibrium value of the gene frequency, $\hat{q}$, when this equilibrium is stable, and minimized when it is unstable. Unfortunately, in order to make this generalization, it was necessary in most cases to define average fitness in a way which is not intuitively obvious. In addition, for the case of an equilibrium due to a balance of selection and mutation, the adaptive value of the population is not quite maximized. Despite these reservations, however, the principle of maximization of adaptive value does, in some sense, tie together the diverse conditions of equilibrium. It is interesting to note the parallel between LI's approach and the conditions for equilibrium of a physical particle subjected to various forces. For such a particle the condition of stable equilibrium is that of minimum potential energy, while unstable equilibrium is characterized by maximum potential energy.

A further, and extremely important, contribution to the general theory of genetic equilibria has been made by KIMURA (1956) in which that writer has developed general conditions for the stability of equilibria when more than two alleles exist at a locus. The restriction on these general conditions is that the selective forces be constant, irrespective of gene frequency, and that mating be at random.

In this paper I will propose a further extension of the general treatment of gene frequency equilibria in a different direction than did KIMURA. While he held selection and mating system constant and allowed the number of alleles to be n, I shall deal with only two alleles, but allow the factors acting on the gene frequency to have the widest possible scope. The methodology developed will

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apply irrespective of the nature of selection, the mating system, the presence of mutation or migration, or the normality of Mendelian segregation.

To this end, a slightly different approach to finding the equilibrium value, $\hat{q}$, will be used, and it will be shown that with this method of attack, the general conditions for stability take an extremely simple form.

To complete the deterministic theory of gene frequency equilibria, it remains to draw together the n-dimensional generalization of KIMURA, with this treatment, a problem which, to this moment, has not been solved.

The method of weights

Let us assume that there are two alleles at a locus, a and A, and that in any particular generation they are in the relative proportions q_0 and $p_0 = (1-q_0)$. In the next generation the three genotypes AA, Aa, and aa will be present in the relative frequencies:

	AA	Aa	aa
(1)	$U_{11}\, p_0^2$	$U_{12}\, 2\, p_0 q_0$	$U_{13}\, q_0^2$

This will be true *irrespective of the mating system*, mode of selection, mutation, migration, or any other force acting on the population. The U's are not to be confused with the adaptive value, W, used by LI. The U's are simply an arbitrary set of weights chosen in order to make expression (1) true. Thus, despite the superficial resemblance of (1) to the distribution of genotypes in a random mating population under selection, it is actually a perfectly general statement. These weights will generally be a function not only of the selection coefficients, mutation rates, migration, and inbreeding coefficient of the population, but also of q itself.

Now the gene frequency of allele a in this next generation is:

$$(2) \qquad q_1 = \frac{p_0 q_0\, U_{12} + q_0^2\, U_{22}}{p_0^2\, U_{11} + 2\, U_{12}\, p_0 q_0 + U_{22}\, q_0^2}$$

and the change in gene frequency from the initial generation is

$$(3) \qquad q_1 - q_0 = \Delta q = \frac{p_0 q_0\, [(U_{12} - U_{11}) + q_0\, (U_{11} + U_{22} - 2\, U_{12})]}{p_0^2\, U_{11} + 2\, U_{12}\, p_0 q_0 + U_{22}\, q_0^2}$$

By equilibrium of gene frequency is meant simply the value or values of q for which $\Delta q = 0$. Setting $\Delta q = 0$ in equation (3) there are three possible solutions. Two of these ($\hat{q} = 1, \hat{q} = 0$) are trivial. We are not concerned with loss or fixation of the allele. The third solution is given by

$$(4) \qquad \hat{q} = \frac{U_{11} - U_{12}}{U_{11} + U_{22} - 2\, U_{12}}$$

This expression (4) is analogous to LI's equation (4) which is simply a special case when the weights are the adaptive values of the three genotypes.

By substitution of the appropriate weights in (4), the value or values of q for which there is an equilibrium can be immediately found.

It was stated at the outset that only the case of two alleles at a locus was being considered here. In the last section of this paper the most general expression for the stability of equilibria involving n alleles will be given. It still remains to translate this general condition into restrictions upon the weights themselves.

The stability of equilibria

By analogy to the equilibrium of mass points in physics, we may classify equilibria as stable, unstable, or neutral. In a stable equilibrium, the gene frequency will return to the equilibrium point, $\hat{q}$, if it is displaced slightly in the positive or negative direction. The stipulation of a *slight* disturbance is necessary, for if several equilibria exist a large disturbance from one equilibrium point may cause the frequency to change toward another equilibrium. Mathematically we may say that the equilibrium is stable if $\left.\frac{d\,\Delta q}{d\,q}\right|_{\hat{q}}$ is negative.

Conversely, the equilibrium is unstable if the gene frequency continues to move away from the equilibrium point following a slight displacement. This may be symbolized as $\left.\frac{d\,\Delta q}{d\,q}\right|_{\hat{q}}$ is positive.

Finally, the equilibrium is neutral if the gene frequency moves neither toward nor away from equilibrium following a small displacement, but remains at the point to which it has been displaced. This means that $\left.\frac{d\,\Delta q}{d\,q}\right|_{\hat{q}}$ is zero.

It may happen that $\frac{d\,\Delta q}{d\,q}$ does not exist at the point $\hat{q}$. There are a great many functions for which this may be true. When such is the case, a slightly more general set of criteria may be useful. Let δq be an increment of q taken to be arbitrarily small. Then the conditions are:

(5a) stable: $\Delta\,(\hat{q}+\delta q) < 0 < \Delta\,(\hat{q}-\delta q)$
(5b) unstable: $\Delta\,(\hat{q}+\delta q) > 0 > \Delta\,(\hat{q}-\delta q)$
(5c) neutral: $\Delta\,(\hat{q}+\delta q) = 0 = \Delta\,(\hat{q}-\delta q)$

In certain complex cases it may be most convenient, simply to graph Δq in the region of $\hat{q}$ and see which of these relations holds .

It has been called to my attention by DR. A. R. G. OWEN of Cambridge, that the criterion of stability, involving only the sign of $\left.\frac{d\,\Delta q}{d\,q}\right|_{\hat{q}}$ as it does, might not suffice since the absolute magnitude of this derivative also seems to affect the nature of the equilibrium. As the following demonstration proves, the absolute

magnitude of $\left.\frac{d\,\Delta q}{d\,q}\right|_{\hat{q}}$ in no way complicates the problem.

Let us assume that at a given instant the gene frequency is at the value of q^*. Then the various possibilities for $\left.\frac{d\,\Delta q}{d\,q}\right|_{q^*}$ may be written as:

(6a) $0 < \left.\frac{d\,\Delta q}{d\,q}\right|_{q^*}$

(6b) $0 = \left.\frac{d\,\Delta q}{d\,q}\right|_{q^*}$

(6c) $-1 \leq \left.\frac{d\,\Delta q}{d\,q}\right|_{q^*} < 0$

(6d) $-2 < \left.\frac{d\,\Delta q}{d\,q}\right|_{q^*} < -1$

(6e) $-2 = \left.\frac{d\,\Delta q}{d\,q}\right|_{q^*}$

(6f) $\left.\frac{d\,\Delta q}{d\,q}\right|_{q^*} < -2$

Condition 6a represents *no equilibrium* (instability) for after a perturbation of q away from q^* the gene frequency will continue to move away from q^* in the same direction as the initial change.

Condition 6b is a state of *neutrality* since there is no further change of q either away from or toward q^* after the initial perturbation.

Condition 6c states that if q is moved away from q^* it will return toward q^* but not pass it. q^* is then a point of *stable equilibrium.*

Condition 6d represents a state of *damped oscillation.* After a change of q, the gene frequency will return toward q^* but will pass it, coming to rest at a new value of q which deviates less in absolute magnitude from q^* than did the initially perturbed value of q. The result of such a process is an oscillating return to q^* by an infinite series of positive and negative steps. Strictly speaking q^* in such a case is a point of *stable equilibrium.*

Conditions 6e and 6f represent, respectively, *uniform* and *increasing* oscillation around q^*. Then at q^*, Δq is not zero because q will pass through q^* and move to a new position deviating more than, or as widely from q^* as did the initially perturbed value. But $\hat{q}$, the equilibrium value, has been defined as the value of q for which Δq is zero. Than any q^* for which $\left.\frac{d\,\Delta q}{d\,q}\right|_{q^*} \leq -2$ is not $\hat{q}$. Conversely, if there is any value of q for which $\Delta q = 0$ neither conditions (6e) or (6f) can apply to it. The only possible conditions on the derivative at $\hat{q}$ are, thus, the first four, which accord completely with the sign criterion of stability.

Having established that the condition $\left.\frac{d\,\Delta q}{d\,q}\right|_{\hat{q}} < 0$ is a necessary and sufficient criterion of stability, we may now convert this inequality into restrictions on the weights themselves.

Restrictions on the weights

Defining

(7a) $a = U_{11} - U_{12}$

(7b) $b = U_{22} - U_{12}$

(7c) $\bar{U} = p^2 U_{11} + 2pq\, U_{12} + q^2 U_{22}$

then from (3)

$$(8) \qquad \Delta q = \frac{pq\,[-a + q\,(a+b)]}{\bar{U}} .$$

Noting that at equilibrium $q\,(a+b) - a = 0$ and that $\bar{U}$ and pq are always positive quantities, the requirement that $\left.\frac{d\,\Delta q}{d\,q}\right|_{\hat{q}} < 0$ is equivalent [from (8)] to the condition

$$(9) \qquad \frac{d}{d\,q}\,[q\,(a+b) - a] < 0 .$$

This expression is identical with the requirement that $\frac{d^2\,\bar{U}}{d\,q^2}$ be negative when the weights are constant since the expression in brackets is the first derivative of $\bar{U}$ for constant selection. For selection which is functionally dependent upon gene frequency, this bracketed expression is easily shown to be equivalent to

$$(10) \qquad \frac{d\,\overline{w}}{d\,q} - \overline{\left(\frac{d\,w}{d\,q}\right)}$$

which is Li's expression (17).

On simplification (9) becomes

$$(11) \qquad 0 > (\hat{a} + b)\left[1 - \frac{d}{d\,q}\left(\frac{a}{a+b}\right)\right]_{\hat{q}}$$

The stability of the equilibrium, then, depends upon the sign of the two quantities $(\hat{a} + b)$ and $1 - \left.\frac{d}{d\,q}\left(\frac{a}{a+b}\right)\right|_{\hat{q}}$. Ignoring for the moment the differentiated function, the condition on the sign of $(\hat{a} + b)$ is exactly that given by KIMURA and LI for constant selective values. Since $\hat{q}$ must be a positive quantity in the interval 0, 1 for a nontrivial solution, $\hat{a}$ and b must be of the same sign. Still ignoring the differentiated quantity, the condition for stability would then be:

$$(12) \qquad \begin{aligned} \hat{a} &= U_{11}(\hat{q}) - U_{12}(\hat{q}) < 0 \\ b &= U_{22}(\hat{q}) - U_{12}(\hat{q}) < 0 \end{aligned}$$

That is, at equilibrium the heterozygote must have a higher fitness than either homozygote.

For the general case in which the weights are functionally related to q, however, the differential quantity cannot be ignored and its sign and magnitude become important. The most general conditions for a stable equilibrium are then (i) The heterozygote is greater in fitness than either homozygote at equilibrium

in which case the slope of the line $f(q) = q - \frac{a}{a+b}$ must be positive at $\hat{q}$

or else

(ii) The heterozygote is lower in fitness than either homozygote in which case the slope of the line $f(q) = q - \frac{a}{a+b}$ must be negative at $\hat{q}$.

It should again be noted that in deriving (11) it was assumed that $\frac{d\,\Delta q}{d\,q}$ existed at $\hat{q}$. There is a set of functions for which this is not true so that recourse must be had to the more primitive conditions 5a, 5b and 5c. A case of this sort will be reviewed in the next section.

Two simple examples will show that an unstable equilibrium may exist even when the fitness of the heterozygote is greater than either homozygote at equilibrium, and that a stable equilibrium may result despite the inferiority of the heterozygote. To demonstrate the first case, consider the weights:

$$U_{11} = 1 \qquad U_{12} = 2q^2 + 1 \qquad U_{22} = 4q^2;$$

from (4)

$$\hat{q} = \frac{\hat{a}}{\hat{a}+b} = 2\hat{q}^2 \tag{13a}$$

$$\hat{q} = \frac{1}{2} \tag{13b}$$

At equilibrium, then, the fitnesses of the genotypes are:

AA	Aa	aa
1.0	1.5	1.0

but

$$1 - \frac{d}{d\,q}\left(\frac{a}{a+b}\right)\Bigg|_{\hat{q}} = 1 - 4\hat{q} = -1 \tag{14}$$

so that

$$(\hat{a}+b)\left(1 - \frac{d}{d\,q}\;\frac{a}{a+b}\right)\Bigg|_{\hat{q}} = (-1)(-1) = 1 \tag{15}$$

The equilibrium is thus an *unstable* one.

The second case can be illustrated by the weights

AA	Aa	aa
1	$1-2q^2$	$2-4q^2$.

Again $\hat{q}$ is ½ so that at equilibrium the fitnesses of the genotypes are:

AA	Aa	aa
1.0	0.5	1.0

and the heterozygote is inferior. The fact that $(\hat{a}+b)$ is equal to unity but $1 - \frac{d}{d\,q}\;\frac{a}{a+b}\Big|_{\hat{q}}$ is again equal to -1 so that $(\hat{a}+b)\left(1 - \frac{d}{d\,q}\;\frac{a}{a+b}\Big|_{\hat{q}}\right) = (1)(-1) = -1$, shows the equilibrium to be *stable*.

Determination of the weights

The determination of the weights is generally a very simple matter and will be illustrated for some cases which demonstrate the generality of the method. The cases chosen are those which have already been attacked by other methods and whose results are known, providing some sort of check on the procedure.

(a) *Inbred population with constant selection:* Under certain conditions a population may reach an equilibrium under inbreeding different from complete homozygosis. For example, a population of plants, a proportion, K, of which are outpollinated and a proportion $(1-K)$ of which are selfed will reach an equilibrium value of F, the coefficient of inbreeding, such that $F = \dfrac{1-K}{1+K}$.

In an inbred population at equilibrium with respect to inbreeding, the zygotes forming the second generation will not be in the ratio $p_0^2 : 2p_0q_0 : q_0^2$. As WRIGHT shows, the zygotic ratio may be put in the form

(16) $\quad (1-F)p_0^2 + Fp_0 \quad : \quad 2p_0q_0(1-F) \quad : \quad (1-F)q_0^2 + Fq_0$

where F is the equilibrium inbreeding coefficient. After selection, then, the three genotypes are in the relative frequencies

$$\begin{matrix} AA & & Aa & & aa \end{matrix}$$

(17) $\quad W_{11}[(1-F)p_0^2 + Fp_0] \quad W_{12}(1-F)\,2p_0q_0 \quad W_{22}[(1-F)q_0^2 + Fq_0]$

Now by factoring out the appropriate quantities from each of these expressions, distribution (17) may be rewritten as:

$$\begin{matrix} AA & & Aa & & aa \end{matrix}$$

(18) $\quad W_{11}\left(1-F+\dfrac{F}{p_0}\right)p_0^2 \quad W_{12}(1-F)\,2p_0q_0 \quad W_{22}\left(1-F+\dfrac{F}{q_0}\right)q_0^2$

These are in the form given by (1) and

$$\begin{aligned} U_{11} &= W_{11}\left(1-F+\frac{F}{p_0}\right) \\ U_{12} &= W_{12}\,(1-F) \\ U_{22} &= W_{22}\left(1-F+\frac{F}{q_0}\right). \end{aligned}$$

Substituting these values in the basic equation (4), and solving for $\hat{q}$, yields:

(19) $\quad \hat{q} = \dfrac{(1-F)(W_{11}-W_{12}) + F(W_{11}-W_{22})}{(1-F)[(W_{11}-W_{12}) + (W_{22}-W_{12})]}$

which is identical with LI's solution (21), in his 1955 paper.

Expansion of $\dfrac{d}{dq}\left.\dfrac{a}{a+b}\right|_{\hat{q}}$ shows that it is always a number smaller than unity, thus assuring that $1 - \dfrac{d}{dq}\left.\dfrac{a}{a+b}\right|_{\hat{q}}$ is always positive.

The conditions for stability then become:

(20) $\quad 0 > W_{11}\left(1-F+\dfrac{F}{\hat{p}}\right) - W_{12}(1-F)$

and

$$(21) \qquad 0 > W_{22}\left(1-F+\frac{F}{\hat{q}}\right) - W_{12}\,(1-F)$$

or their equivalents:

$$(22) \qquad 0 > (W_{11}-W_{12})\,(1-F) + \frac{F\,W_{11}}{\hat{p}}$$

$$(23) \qquad 0 > (W_{22}-W_{12})\,(1-F) + \frac{F\,W_{22}}{\hat{q}}$$

In order that relations (22) and (23) hold it is necessary that the heterozygote be superior to both homozygotes. This is not sufficient however. Rearrangement of (22) and (23) yields

$$(24) \qquad \hat{p} > \frac{F}{1-F}\left(\frac{W_{11}}{W_{12}-W_{11}}\right)$$

$$(25) \qquad \hat{q} > \frac{F}{1-F}\left(\frac{W_{22}}{W_{12}-W_{22}}\right)$$

which by addition results in

$$(26) \qquad \frac{1-F}{F} > \frac{W_{11}}{W_{12}-W_{11}} + \frac{W_{22}}{W_{12}-W_{22}}$$

This last requirement, together with that of overdominance defines stability.

(b) *Compensation:* An excellent illustration of variable selection coefficients is provided by the phenomenon of *compensation*. By compensation is meant the production of a relatively greater number of offspring by certain matings. This is not the same as simple selection since the differential fecundity is a function of the mating combination, rather than of the genotypes of the parents alone. Li (1953) dealt with the case of selection against heterozygotes, accompanied by compensation in those matings which would produce heterozygotes. Homozygous recessive females when mated either to heterozygous or homozygous dominant males, produce $(1+t)$ offspring for each offspring produced by other matings. Here t is the compensation coefficient. The assumption is made that for the selection coefficients $W_{11}=W_{22}=1$ and $W_{12}=(1-s)$. Li then shows that the relative frequencies of the three genotypes following selection and compensation are:

$$(27) \qquad \begin{array}{ccc} AA & Aa & aa \\ p_0^2 & \left(1-\frac{s\,q_0}{2}\right) 2\,p_0\,q_0 & (1+t\,p_0\,q_0)\,q_0^2 \end{array}$$

On substituting these weights into the basic equation (4) we find that

$$(28) \qquad \hat{q} = \frac{t+s-\sqrt{t^2+s^2}}{2\,t}$$

which is identical with Li's solution. By inspection of the weights, it is clear that for all $q \neq 0$

$$(29a) \qquad U_{11} > U_{12} < U_{22}$$

In addition

$$(29b) \qquad \frac{d}{dq}\left.\frac{a}{a+b}\right|_{\hat{q}} = \frac{2st}{(s+t+\sqrt{t^2+s^2})^2}$$

which is smaller than unity for all s, t. The equilibrium is then, *unstable*, as concluded by LI (1953).

LEWONTIN (1953) investigated the equilibrium for the case of compensation and selection for a dominant gene. Using LI's notation the weights are found to be

$$(30) \qquad \begin{aligned} U_{11} &= (1-s)(1+t) \\ U_{12} &= (1-s)(1+t) \\ U_{22} &= 1+t(1-q^2) \end{aligned}$$

Now, since $U_{11} = U_{12}$ for all q, the only possibility of equilibrium is when

$$(31) \qquad U_{11}(\hat{q}) = U_{12}(\hat{q}) = U_{13}(\hat{q})$$

The only value of q which satisfies (31) is

$$(32) \qquad \hat{q} = \sqrt{\frac{s(1+t)}{t}}.$$

Moreover, this is a stable equilibrium since q will increase if $U_{11}(q) = U_{12}(q) < U_{22}(q)$ while it will decrease if $U_{11}(q) = U_{12}(q) > U_{22}(q)$.

These are equivalent to

$$(33a) \qquad \text{Increase of } q: \quad q < \sqrt{\frac{s(1+t)}{t}}$$

$$(33b) \qquad \text{Decrease of } q: \quad q > \sqrt{\frac{s(1+t)}{t}}$$

This solution by inspection is simply a special application of condition (5a) above, and is necessary because $\dfrac{d}{dq}\left(\dfrac{a}{a+b}\right)\Big|_{\hat{q}}$ does not exist.

(c) *Selection and mutation:* The simplest form of balance between selection and mutation will be considered. It is assumed that the homozygous recessive genotype is selected against. Its adaptive value is $W_{22} = (1-s)$. At the same time A mutates to a at a rate μ per generation. If p_0 and q_0 are the gene frequencies in the initial generation, then the relative frequencies of the gametes will be $p_0 - \mu p_0$ and $q_0 + \mu p_0$ for A and a respectively.

Following random union of gametes and selection, the relative frequencies of the genotypes in the next generation will be:

$$(34) \qquad \begin{matrix} AA & Aa & aa \\ p_0^2(1-\mu)^2 & 2p_0q_0(1-\mu)[q_0(1-\mu)+\mu] & [q_0(1-\mu)+\mu]^2 W_{22} \end{matrix}$$

On factoring out the appropriate terms in each entry we have:

$$(35) \qquad \begin{matrix} AA & Aa & aa \\ p_0^2(1-\mu)^2 & 2p_0q_0\left[(1-\mu)\left(1-\mu+\dfrac{\mu}{q_0}\right)\right] & q_0^2\left[1-\mu+\dfrac{\mu}{q_0}\right]^2(1-s) \end{matrix}$$

Substituting the weights given by (35) into equation (4) and solving for $\hat{q}$ yields

$$\hat{q} = \frac{1}{1-\mu}\left(\sqrt{\frac{\mu}{s}} - \mu\right) \tag{36}$$

This solution differs slightly from that given by LI for two reasons. The model we have used is slightly more exact since LI assumes selection and mutation to act simultaneously on the same gene frequency q, while we assume selection to act on the altered gene frequencies arising from mutation. In addition LI rejects higher powers of μ and assumes $(1 \pm \mu)$ to be not different from unity. For the usual mutation rates postulated of the order of 10^{-5} these assumptions are quite reasonable. Applying them to equation (36) we have

$$q \cong \sqrt{\frac{\mu}{s}} \tag{37}$$

which is identical with that given by LI, and is the form usually accepted. Now it is clear that for all values of q,

$$0 > U_{11} - U_{12} \tag{38}$$

moreover

$$0 > U_{22} - U_{12} \tag{39}$$

if $s > \mu$. In order for there to be any nontrivial equilibrium the selection against the gene must be greater than the mutation rate.

Moreover this equilibrium is stable since, for $s > \mu$, $\hat{a} + b < 0$ and

$$\frac{d}{dq}\left(\frac{a}{a+b}\right)\Bigg|_{\hat{q}} = \frac{-(1-\mu)^2\,\mu\,(s-\mu)}{[\mu\,(1-\mu) + (s-\mu)\,(q-\mu q+\mu)]^2} < 0$$

(d) *Abnormal segregation ratios:* To complete the picture of the generality of equation (4) as a means of finding equilibrium conditions, it is interesting to apply the method of weights to the case of abnormal segregation of genes. DUNN (1953) has described a highly aberrant condition in mice in which males heterozygous for a recessive allele, t, do not produce t and + gametes in a normal 1:1 ratio. In an accompanying article PROUT (1953) demonstrated that this aberrant segregation ratio could lead to an equilibrium when the various genotypes were differentially selected. PROUT's result applies only when the segregation abnormality is present in both sexes. Let

q_0 = frequency of t alleles in the gametic pool after segregation
$1 - r = W_{11}$
$1 - s = W_{22}$

and m = proportion of t alleles among the gametes produced by heterozygotes.

Then, following random mating and selection, the three genotypes will be in the proportions

	+/+	+/t	t/t
(40)	$p_0^2\,(1-r)$	$2\,p_0\,q_0$	$q_0^2\,(1-s)$

Following selection, segregation will take place. The gametes will then be in the proportion

$$\begin{matrix} & + & & t \\ (41) \qquad & p_0^2(1-r) + 2\,p_0\,q_0\,(1-m) & & 2\,p_0\,q_0\,m + q_0^2\,(1-s) \end{matrix}$$

Unlike the previous examples, the application of a complete cycle of selection and segregation, results in ratios of the gametes, not the zygotes. The weights of the zygotes are very simply determined, however, by finding what weights could have been applied to the zygotes in order to yield the gametic ratios (41) if segregation had been normal. This is done by adding and subtracting the quantity $4\,p_0q_0\,m$ in the frequency of + gametes. This yields

$$\begin{matrix} & & + & & t \\ (42) \qquad & p_0^2\,(1-r) + 2\,p_0\,q_0 + 2\,p_0\,q_0\,m - 4\,p_0\,q_0\,m & & 2\,p_0\,q_0\,m + q_0^2\,(1-s) \end{matrix}$$

But this would be the gametic ratio resulting from normal segregation had the zygotes been in the frequency

$$\begin{matrix} & +/+ & +/t & t/t \\ (43) \qquad & p_0^2\,(1-r) + 2\,p_0\,q_0\,(1-2\,m) & 4\,p_0\,q_0\,m & q_0^2\,(1-s) \end{matrix}$$

Factoring out the appropriate terms in each entry the weights are seen to be

$$\begin{aligned} U_{11} &= 1-r+2\frac{q_0}{p_0}\,(1-2\,m) \\ U_{12} &= 2\,m \\ U_{22} &= 1-s \end{aligned}$$

Substituting these weights in the basic formula (4) yields

$$(44) \qquad \hat{q} = \frac{r+2\,m-1}{s+r}$$

as a solution. This is identical with PROUT's result.

Thus the method of weights is applicable not only irrespective of the mating system and the form of selection, but also of any assumption about the normality of the underlying Mendelian segregation.

The various combinations of s, r and m which satisfy the stability requirement are too numerous and complex to present. It is sufficient to observe that the numerical cases discussed by PROUT in his original paper all satisfy conditions (11) and so are stable.

Multiple alleles

Suppose that at any locus there are n alleles a_i each present in the the frequencies q_i. Then in a manner entirely analogous to the case of two alleles the genotype frequencies can be written as:

$$\begin{aligned} a_i\,a_i &: \quad U_{ii}\,q_i^2 \qquad\qquad i = 1,2,3,\cdots n \\ a_i\,a_j &: \quad 2\,U_{ij}\,q_i\,q_j \qquad i<j = 2,3,4\cdots n \end{aligned}$$

LI (1955) and WRIGHT (1949) show quite simply that

$$(45) \qquad \Delta\,q_i = \frac{q_i}{\bar{U}}\,(U_{i\cdot}-\bar{U})$$

where the U are constants but this applies equally well to variable weights. In expression 45

$$U_{i\cdot} = \sum_{j=1}^{n} q_j U_{ij} \tag{46}$$

and

$$\bar{U} = \sum_{i=1}^{n} q_i U_{i\cdot} \tag{47}$$

That is, $U_{i\cdot}$ is the average weight for allele a_i in all its combinations and $\bar{U}$ is the grand average weight of the population.

At equilibrium $\Delta q_i = 0$ for all i. There are $n-1$ independent equations of the form $U_{i\cdot} - \bar{U} = 0$ and the solution to these simultaneous equations can easily be shown to be the determinantal equations:

$$\hat{q}_i = \frac{D_i}{D} \tag{48}$$

where

(49)

$$D_i = \begin{bmatrix} (U_{1,1}-U_{n,1}) & (U_{1,2}-U_{n,2}) & \cdots & (U_{1,n-1}-U_{n,n-1}) \\ (U_{2,1}-U_{n,1}) & (U_{2,2}-U_{n,2}) & \cdots & (U_{2,n}-U_{n,n-1}) \\ \vdots & \vdots & & \vdots \\ \vdots & \vdots & & \vdots \\ \vdots & \vdots & & \vdots \\ (U_{n-1,1}-U_{n,1}) & (U_{n-1,2}-U_{n,2}) & \cdots & (U_{n-1,n-1}-U_{n,n-1}) \end{bmatrix}$$

except that the ith column is replaced by the elements:

$$\begin{bmatrix} (U_{n,n}-U_{1,n}) \\ (U_{n,n}-U_{2,n}) \\ \vdots \\ \vdots \\ \vdots \\ (U_{n,n}-U_{n-1,n}) \end{bmatrix}$$

and

$$D = \sum_{i=1}^{n} D_i \tag{50}$$

It should be remembered that $U_{ij} = U_{ji}$. This is the most general form of the determinatal equations which have been given by LI (1955) for the case of $n = 3$ and constant selective values.

The stability of equilibria involving more than two alleles where the U's are constant has been discussed, as I have already pointed out, by KIMURA. When the U's are functions of gene frequency, however, KIMURA's rules do not apply although a general expression can be derived using the same logic as KIMURA.

Let $\hat{Q}$ represent a point of equilibrium in n-1 dimensional space (corresponding to the n-1 independent gene frequencies) given by equations (48), and let $\delta\hat{Q}$ be a

vector representing a small displacement of gene frequencies away from $\hat{Q}$ to a new point Q^*. If $\hat{Q}$ is a point of stable equilibrium there will be a new change in gene frequencies from Q^* back toward $\hat{Q}$ which change can be represented by a vector ΔQ^*. What is meant by *back toward* $\hat{Q}$ is best illustrated in Figure 1. An n-1 dimensional sphere is drawn with $\hat{Q}$ at its center and $\delta\hat{Q}$ as its radius. Then if $\hat{Q}$ is a point of stable equilibrium ΔQ^* will carry the gene frequency to a point Q^{**} which lies *within the sphere*. In order for this to be true, two require-

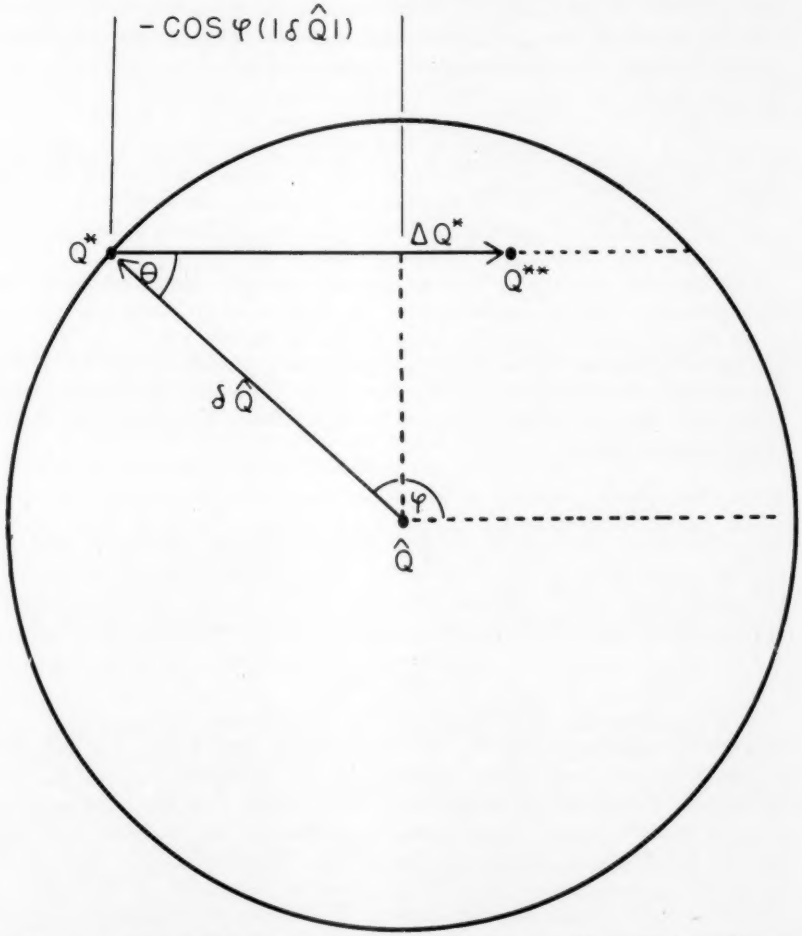

FIGURE 1.—Geometrical representation of the conditions for a stable equilibrium with more than two alleles at a locus. See text for explanation of symbols.

ments must be satisfied. First, the angle θ must be strictly less than 90° which is equivalent to requiring that the angle ϕ between the two vectors be strictly greater than 90°. Second, the vector ΔQ^* must not be too long or it will carry Q^{**} outside of or exactly on to the sphere, no matter what the angle θ.

The cosine of an angle between two vectors is defined as the ratio of the inner product of those vectors to the product of their lengths. The requirement that ϕ be greater than 90° can then be written as:

$$(51) \quad \cos\phi = \frac{|\Delta Q^* \cdot \delta\hat{Q}|}{|\Delta Q^*| \cdot |\delta\hat{Q}|} < 0$$

The requirement that the length of the vector ΔQ^* be short enough to insure that Q^{**} lies strictly within the sphere is equivalent to

$$(52) \quad \frac{|\Delta Q^*|}{|\delta\hat{Q}|} < -2\cos\phi$$

by the simple geometry of Figure 1. Combining (51) and (52) gives the most general requirement for stability

$$(53) \quad 0 > \frac{|\Delta Q^*|^2}{|\Delta Q^* \cdot \delta\hat{Q}|} > -2$$

Condition (51) is given by KIMURA and condition (52) appears to be his condition that no "cyclically winding paths" are involved. A cyclically winding path in n-1 dimensions is the equivalent of an oscillating equilibrium in one dimension and while we showed that oscillation around $\hat{q}$ is not possible by definition in the one dimensional case, this is not true for higher dimensions, so that the second requirement must be added. If expression 53 is reduced to the one dimensional case it becomes simply

$$(54) \quad 0 > \frac{d\Delta q}{dq}\bigg|_{\hat{q}} > -2$$

which was shown earlier to be precisely the condition for stability (6c and 6d).

Whereas condition 53 can be written in terms of the weights in any specific case, I have been unable to find a general restriction on the weights analogous to (11) so that for the present, at least, it is necessary to treat each case specially.

DISCUSSION

As was pointed out at the beginning, the methodology described here is designed as a constructive tool for investigating the equilibrium condition of a population under a wide variety of situations. The examples given merely serve to illustrate the power and generality of the method. It is becoming apparent that the adaptive values of genotypes in populations are functions, and often complex functions, of the gene frequency. This is not surprising since the environment of an organism includes the relative frequencies of other sorts of organisms in the population (See LEVENE, PAVLOVSKY and DOBZHANSKY 1954; LEWONTIN 1955). As various new situations arise, the method may be used in investigation of the equilibrium conditions.

Perhaps the most suggestive result of the mathematical analysis is the demonstration that stable equilibria may exist despite an inferiority of the heterozygote, provided that the adaptive values of the genotypes change properly with gene frequency. Whether evolution does in fact produce such balanced, nonheterotic, polymorphisms is problematical, but their existence is at least possible.

Whereas a general theory of equilibria encompassing adaptive values functionally related to gene frequency on the one hand and multiple allelism on the other is a step in the direction of increased reality of our models of population dynamics, there are a number of areas for theoretical study which are yet untouched or virtually so, but which are essential to a proper understanding of natural events. Among the most important are a study of general equilibrium conditions for many loci interrelated by linkage and epistasis, and the problem of environments which vary both in time and in space. This latter problem demands a revision of our static concept of equilibrium to include the idea of a dynamic and ever fluctuating genetic structure of a population. It is impossible to say whether such problems will ever be amenable to general mathematical analysis, but if population geneticists are to lay any claim to an understanding of the forces molding the genetic structure of natural populations, attempts upon these problems must be made.

SUMMARY

A basic method has been suggested for investigating the equilibrium condition of gene frequency in populations. The method is applicable to any population irrespective of the nature of the forces which change gene frequency. It applies equally well to random and nonrandom mating schemes provided an equilibrium condition of inbreeding can be specified, and irrespective of any assumptions about the regularity of Mendelian segregation at meiosis. It cannot be applied to more than one locus if epistasis is present nor is it useful if the various forces change over time in a way unrelated to gene frequency.

Within these limits it has been shown that superiority in fitness of the heterozygote is neither a necessary nor sufficient condition for equilibrium but that nonheterotic balanced polymorphic systems may exist. Conversely heterotic systems do not lead ineluctably to stable equilibria.

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A STABLE SOMATIC MUTATION TO COLORLESS FROM VARIEGATED PERICARP IN MAIZE

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COLORLESS pericarp and cob is one of the least frequent mutant phenotypes among the offspring of maize plants carrying the somatically unstable variegated pericarp allele, P^{vv}. BRAWN (1956), for example, found only eight mutations to colorless pericarp and cob in contrast to 125 to red pericarp and cob (P^{rr}) among 4575 offspring from the mating homozygous variegated times standard colorless ($P^{vv}\ P^{vv} \times P^{wiw}$) in the W22 inbred line with which we are here concerned.

Tests show that some apparently colorless mutants actually are very low grade variegateds, in that they occasionally show one or more, usually very small, colored stripes in the pericarp. Evidently others are highly stable for colorless. The present study is concerned with a mutant of this latter type. The case is of interest in demonstrating that Modulator, which ordinarily is a readily transposable factor, may become firmly bonded at the *P* locus, resulting in the formation of an isoallele of P^{wiw}, the commonly occurring highly stable colorless form.

ORIGIN OF THE COLORLESS MUTANT

The colorless mutant, termed $P^{wiw}_{m\text{-}1}$, appeared originally as a 43-kernel patch on an otherwise typical medium variegated pericarp ear bearing about 320 seeds. The plant in question was heterozygous for colorless pericarp, red cob (P^{wir}) and belonged to the first backcross generation of a variegated stock being graded to a commercial-type P^{wir} inbred line, known as W22. The mutant ear, in turn, was pollinated by W22.

The original mutant kernels were weakly colored in the silk attachment region, rather than entirely colorless. This pigmentation is attributable to "dark crown," a rather common, nonheritable manifestation of P^{vv} in the pericarp epidermis (EMERSON 1917, 1929). It did not recur among the descendants of $P^{wiw}_{m\text{-}1}$. Seventeen plants were reared from mutant seeds, all of which had entirely colorless pericarp. Pollen from 16 of these individuals was placed on W22 ear shoots, and the plants themselves were pollinated with a homozygous P^{vv} stock. The resulting progenies showed that two equally frequent genotypes were represented in the original mutant patch of kernels. Seven of the individuals gave about equal numbers of

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offspring with variegated or red cob in the testcross with $P^{vv}P^{vv}$, and the same proportion with light red ($P^{wo}_{m-1}\,P^{vr}$) or red ($P^{vr}P^{vr}$) cob in the mating with W22. Evidently these seven plants were heterozygous $P^{wo}_{m-1}\,P^{vr}$. The remaining nine sibs proved to be $P^{vr}P^{vr}$ by the same tests. Twenty-two plants grown from seed on the nonmutant portion of the foundation ear were distributed as follows: 14 medium variegated, one light variegated, two self color, and five colorless pericarp, red cob. This is a result expected from a heterozygous $P^{vv}P^{vr}$ plant. One may conclude from these data that the aberrant patch of kernels was due to a somatic mutation of the P^{vv} allele to colorless, occurring fairly early in the ontogeny of the ear. The epidermal layer of the pericarp was not involved in the change of P^{vv} to P^{wo}_{m-1}, as would be expected in view of the separate origin of this structure; it happened to show the so-called dark crown pattern as a result of an independent mutation in this superficial tissue.

PROBLEMS AND PROCEDURES

The first question to which an answer was sought was whether the exceptional stability of the mutant rested upon a deficiency at the *P* locus, giving a null effect for pigmentation. Since all known deficiencies in maize either are not normally transmitted through the pollen or are lethal when homozygous, these criteria were applied in the present case.

The second point to which attention was directed was the degree of stability of the colorless mutant. This led to a search among some 4300 descendants of the mutant for even very small colored sectors on the ears and for plants arising from germinal mutations. Such mutations were looked for in both P^{wo}_{m-1} homozygotes and heterozygotes, and in families varying in genetic background.

The third main problem was concerned with the relation of Modulator (*Mp*) to the colorless mutation. Earlier evidence has led to the view that the variegated pericarp allele is a dual structure comprising the P^{rr} gene and Modulator, the latter suppressing the pigment-producing action of the former when the two elements are in conjunction at the *P* locus. Mutations of variegated to self color (P^{rr}), which occur with frequencies of 3–10 percent in different $P^{vv}P^{vr}$ strains, were shown to involve loss of *Mp* from the *P* locus by selective transposition of the element, usually to another chromosome site (BRINK and NILAN 1952). What is the colorless mutant in terms of P^{rr} and *Mp*? Is P^{wo}_{m-1} a counterpart of ordinary P^{vv} in which Modulator merely has become fixed at the *P* locus, or has *Mp* also undergone a qualitative change? Another possibility was that the colorless phenotype was due to mutation of P^{rr} to the stable P^{wo} colorless allele. This is not a frequent event, but it has been observed in an experimental culture (BRINK 1929). Had it been found that the mutation was due to a change in P^{rr}, then the observed persistence of Modulator at the *P* locus would have had to be accounted for.

Advantage was taken in this part of the study of the fact that Modulator promotes the Dissociation (*Ds*) type of chromosome breakage (BARCLAY and BRINK 1954). Populations may be scored by the *Ds* test for distribution of Modulator in

the absence of an overt effect of the latter element at the *P* locus. Also the *Ds* chromosome breakage pattern varies both with the dosage and qualitative nature of the co-factor present in the genome, Activator, as McCLINTOCK (1951) first showed, or Modulator, as BARCLAY and BRINK (1954) subsequently observed. It was thus possible to characterize Modulator in the P^{wlw}_{m-1} mutant by a criterion independent of the phenotypic effect of *Mp* at the *P* locus. The procedure followed in the *Ds* tests will be described later in the report.

Additional evidence on the relation of Modulator to the colorless mutant was afforded by observing the effect of the P^{ww}_{m-1} allele in heterozygotes with ordinary variegated pericarp. WOOD and BRINK (1956) recently have shown that the pronounced decrease in mutability of the P^{vv} allele in P^{vv} homozygotes, as compared with $P^{vv}P^{wr}$ heterozygotes which EMERSON (1929) first noted, can be explained in terms of dosage action of Modulator. A comparison was made in the present study of the variegation grades of P^{wlw}_{m-1} P^{vv} and $P^{wr}P^{vv}$ sib ears resulting from P^{wlw}_{m-1} $P^{wr} \times P^{vv}P^{vv}$ matings to determine whether the *Mp* component of P^{wlw}_{m-1} retained the capacity to reduce mutability of P^{vv}.

EXPERIMENTAL RESULTS

Transmission of P^{ww}_{m-1}

The mutant colorless allele is transmitted normally through both the female and male gametophytes. Among 1278 offspring from P^{wlw}_{m-1} P^{wr} ♀ × P^{wlw} ♂ matings, for example, 658 had red cobs ($P^{wr}P^{wlw}$) and 620 had white cobs ($P^{wlw}_{m-1}P^{wlw}$). The reciprocal type of cross gave 1693 plants with light red cobs and 1695 with colorless cobs, plus one exceptional individual (see later). The deviations from expectation on a monohybrid basis are well within sampling limits in both these distributions.

Homozygous P^{wlw}_{m-1} plants are indistinguishable in plant and ear size from P^{wr} individuals of the same genetic background.

There is no evidence, therefore, that P^{wlw}_{m-1} adversely affects development either of the gametophytes or of the sporophyte, as would be expected if it represented a deficiency for the *P* locus.

Stability

The uniformly colorless pericarp resulting from the first few testcrosses with the P^{wlw}_{m-1} mutant showed that the latter was remarkably stable, as compared with ordinary variegated. Additional data on stability then were sought from larger scale experiments.

A total of 4330 plants were reared which were heterozygous for P^{wlw}_{m-1} and one or the other of the standard colorless alleles, P^{wr} or P^{wlw}. Among these plants, 553 occurred in families from recurrent backcrosses to the W22 inbred line. The remainder were derived from outcrosses of W22-type, P^{wlw}_{m-1} P^{wr}, individuals to one or another of three uniform, but unrelated P^{wlw} stocks, one of which was the

4Co63 inbred line. The latter was known to give particularly strong expression (high somatic mutation rate) of variegated pericarp when an ordinary P^{vv} allele was introduced into it. In addition to these heterozygotes, 206 plants homozygous for $P^{vtw}_{m\text{-}1}$ also were scored. The ears from all these plants were examined minutely under 2× magnification for red sectors, the scoring usually being checked by a second person.

The pericarp of all 4330 plants carrying the $P^{vtw}_{m\text{-}1}$ allele, with one exception, was colorless. One ear occurred in which there were numerous, very fine, red stripes. The pattern in this case was not typical of any of the commonly encountered phases of variegated pericarp in that all the colored sectors were narrow, rather than of variable widths.

The exceptional, lightly striped, ear occurred in a highly uniform family, 38–101C, resulting from a P^{vtw} ♀ × $P^{vtw}_{m\text{-}1}P^{vr}$ ♂ mating. Since the staminate parent was the heterozygote in the testcross, it is possible that the aberrant individual was a contaminant. This explanation cannot be excluded, because distinctive marker genes were not present and there were other variegated plants in the vicinity from which pollen might have been derived at the time the mating in question was made. It is quite unlikely, however, that the variegated individual resulted from stray pollination. The ear was typical in size and form for the uniform family in which it occurred. Secondly, the variegation pattern was unusual, and not of the sort expected from an ordinary P^{vv} allele, even if accompanied by one or more transposed Modulators. A regular effect of the latter is to suppress most late occurring mutations of P^{vv} to P^{rr}, which are expressed as narrow sectors in the pericarp. Uniqueness of the color pattern and conformity of the ear to family type point to the conclusion that the exceptional plant carried a mutation of the $P^{vtw}_{m\text{-}1}$ allele to an uncommon form of variegated pericarp. Further tests could not be made because the mutant plant, like its sibs, was open pollinated in a section of the nursery in which heavy contamination from other stocks would be expected.

Modulator as a component of the $P^{vtw}_{m\text{-}1}$ *allele*

Tests will now be described the results of which show that Modulator is a component of $P^{vtw}_{m\text{-}1}$, and that the latter regularly assorts as a *P* allele.

Plants with a W22 genotypic background and heterozygous $P^{vtw}_{m\text{-}1}P^{vr}$, were pollinated by a standard colorless (P^{vtw}) inbred stock carrying the marker genes *wx* (waxy endosperm), *y* (white endosperm), and W^c (dominant white capping when *Y* is present). An occasional deep yellow (non-W^c) kernel, probably the result of contamination, was removed from the resulting ears. The F_1 kernels were homozygous for the recessive genes, *c* and *r*, for colorless aleurone. They were planted in an isolated detasseling plot in which the pollen source was *wx y* P^{vtw} *R C* plants, either homozygous or heterozygous for Dissociation (*Ds*). *Ds* in this stock was located in the standard position (McCLINTOCK 1953), proximal to the *C* locus on the short arm of chromosome 9. Had *Ds* not been present the kernels

formed on the detasseled plants all would have been self-colored (or dark mottled) as a result of fertilization of the duplex *cc rr* fusion nuclei of the female gametophytes by *C R* sperm. The *C* chromosome in a large proportion of the male gametes, however, carried *Ds* in a proximal position. This factor, in the form here used, promotes chromosome breakage with high frequency at the *Ds* locus if either Activator (McCLINTOCK 1951) or Modulator (BARCLAY and BRINK 1954) is present in the genome. Such breakage in the present case would give rise to acentric chromosome fragments bearing the *C* gene, which would then be lost to the nucleus. The result of a *C*-loss, in mitotically active endosperm tissue, is a lineage of cells in which the aleurone phenotype is colorless rather than colored. Since such *C* to *c* mottling would occur only in kernels formed by plants carrying Modulator, the procedure serves as a test for distribution of plants carrying *Mp* in the population. *R*-mottling did not interfere significantly in the present material with identification of the two expected classes of plants, those forming kernels with and those without *C* to *c* changes.

A further check on contamination was afforded in this generation by the presence of the *wx* and *y* markers. Any ear which did not segregate for both these genes could not have been of the designated parentage. No such contaminants were found, the prior screening based on dominant white capping (W^c) apparently having removed all of them.

The mature ears borne by the pistillate plants in the detasseling plot were harvested and separated into two groups, red cob and colorless cob. Each ear then was examined for the presence of kernels showing *C* to *c* mottling in the aleurone, the specific reaction denoting presence in the genome of Modulator. The distributions for cob color and *Mp* are summarized in Table 1.

TABLE 1

The distribution of cob color and C *to* c *aleurone mottling resulting from* Ds *action among the offspring from* $P^{ww}_{m-1}P^{wr} \times P^{ww}$ *matings*

Family	Red cob		Colorless cob	
	Mottled	Nonmottled	Mottled	Nonmottled
38–148	0	175	161	0
–149	0	95	107	0
–150	0	81	87	0
–151	0	106	99	0
–152	0	95	105	0
–153	0	60	59	0
–154	0	59	69	0
–155	0	61	50	0
–156	0	115	88	0
–157	0	93	116	0
–158	0	116	101	0
–159	0	107	94	0
–161	0	57	63	0
Totals	0	1220	1199	0

The numerical results show that the colorless mutant behaves as an allele of P^{wr}, as expected from the outcome of earlier tests. Secondly, the 1220 red cob (P^{wr}) segregates, without exception, were negative for *C* to *c* mottling, whereas all 1199 colorless cob offspring were positive for this character. Thus, there was no recombination in the 2419 plants tested between the colorless pericarp mutant phenotype and the capacity to induce *Ds* chromosome breakage. The regular assortment of Modulator with colorless shows that *Mp* is "fixed" at the *P* locus, and may be assumed to be a component of the $P^{ww}_{m\text{-}1}$ allele.

The C *to* c *aleurone mottling pattern associated with the* $P^{ww}_{m\text{-}1}$ *allele*

It is a question of interest whether the Modulator components of $P^{ww}_{m\text{-}1}$ and the P^{vv} parent allele are alike in number and kind. The *Ds* chromosome breakage patterns which they incite provide a basis for comparing them which is independent, presumably, of their local action on the *P* phenotype.

Plants homozygous for P^{ww} and *C Ds*, the latter element again being in standard position proximal to *C*, were pollinated by (1) $P^{vv}P^{vv}$ and (2) $P^{ww}_{m\text{-}1}P^{wr}$ individuals which conformed closely in residual genotype to the W22 inbred line. The P^{vv} allele was from the stock in which the colorless mutant occurred. The triploid endosperm genotypes expected from these matings are as follows:

Mating 1. *C Ds*, P^{ww} ♀ × *c ds* P^{vv} ♂ = *C Ds/C Ds/c ds*, $P^{ww}P^{ww}P^{vv}$

Mating 2. *C Ds*, P^{ww} ♀ × *c ds* $P^{ww}_{m\text{-}1}P^{wr}$ = (a) *C Ds/C Ds/c ds*, $P^{ww}P^{ww}P^{ww}_{m\text{-}1}$

(b) *C Ds/C Ds/c ds*, $P^{ww}P^{ww}P^{wr}$

The (b) kernels from mating 2 lack Modulator, and so would be expected to have self-colored aleurone. Mating 1 and the (a) kernels from mating 2, on the other hand, should show *C* to *c* mottling according to the respective effects on *Ds* chromosome breakage of the Modulator components of the P^{vv} and $P^{ww}_{m\text{-}1}$ alleles. The mottling in these cases would ensue from "double breaks," simultaneous or tandem, at *Ds*, whereby both the *C* genes initially present in the nucleus would be lost on acentric fragments.

The results of the two sets of testcrosses are presented in Figure 1. The middle column of self-colored kernels is the 2(b) class mentioned above. The two columns at the left show the pattern of *C* to *c* aleurone mottling resulting from action on *Ds* of the Modulator present in the parent P^{vv} allele. The two right hand columns illustrate the effect of *Mp* in the $P^{ww}_{m\text{-}1}$ mutant. It is evident from the photograph that there is no discernible difference in the mottling patterns in the two sets of kernels.

Two conclusions follow from these facts: (1) The dosage level of Modulator is the same in the P^{vv} and $P^{ww}_{m\text{-}1}$ alleles and (2) The *Mp* units in the two cases are indistinguishable by this test.

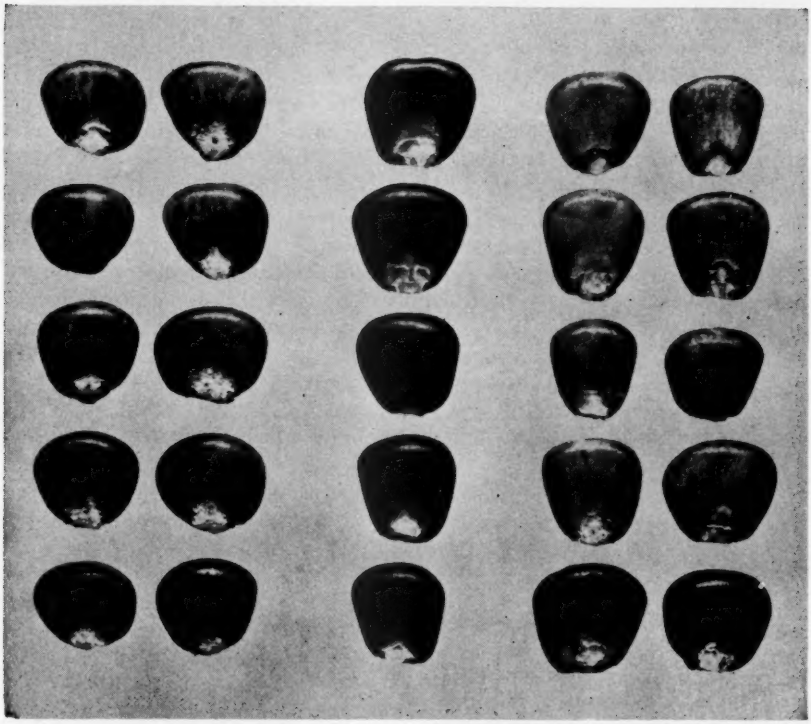

FIGURE 1.—The *C* to *c* aleurone mottling patterns resulting from *Ds* chromosome breaks in response to the presence of a standard medium variegated pericarp allele, P^{vv}, (left), and the colorless pericarp mutant, $P^{ww}_{m\text{-}1}$, (right). The kernels in the middle column lack Modulator, and thus show no aleurone mottling associated with *Ds* chromosome breakage.

Effect of $P^{ww}_{m\text{-}1}$ *on expression of variegated in heterozygotes with an ordinary* P^{vv} *allele*

Each of the P^{vv} alleles in a homozygous $P^{vv}P^{vv}$ plant tends to reduce mutability to self-color of the other. This usually results in a smaller total of such mutations in homozygotes than in plants heterozygous for variegated and a standard stable allele, such as P^{wr}. WOOD and BRINK (1956) showed that Modulator, when transposed from the *P* locus to one or another chromosome site, has the same effect on mutation of P^{vv} in $P^{vv}P^{wr}$ plants as each of the P^{vv} alleles in a $P^{vv}P^{vv}$ individual has on mutation of its homologue. Thus the mutation rate of P^{vv} to P^{rr} is a function of *Mp* dosage.

A test was made in the present study for an effect of P^{wlw}_{m-1} on the mutation frequency of an ordinary P^{vv} allele in $P^{wlw}_{m-1}P^{vv}$ heterozygotes comparable to the reciprocal transallelic action previously observed in $P^{vv}P^{vv}$ homozygotes. $P^{wlw}_{m-1}P^{wr}$ plants in an inbred W22 stock were pollinated by a $P^{vv}P^{vv}$ individual carrying a P^{vv} allele known to give the conventional medium variegated pattern in heterozygotes with P^{wr}. The two classes of offspring from this mating, $P^{vv}P^{wr}$ and $P^{vv}P^{wlw}_{m-1}$, are readily separable in that cob color is red in the first and very lightly variegated in the second (cf. middle ears in Figure 2).

Photographs of the two classes of testcross ears are shown in Figure 2. The pair of $P^{vv}P^{wr}$ ears with red cobs, at the left, are typical medium variegateds, as expected. The two ears at the right, with weakly colored cobs, and carrying the same P^{vv} allele but, in this case, heterozygous for the P^{wlw}_{m-1} mutant rather than P^{wr}, show markedly fewer mutations to P^{rr}. Presumably the reduction is a result of the stabilizing action of the Modulator component of P^{wlw}_{m-1} on the P^{vv} allele. Al-

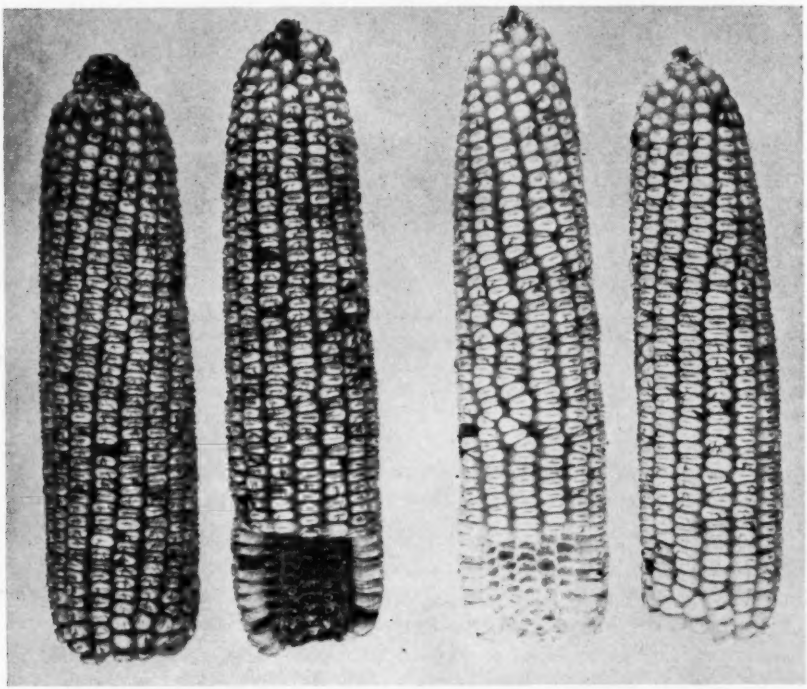

FIGURE 2.—The depressing effect of the colorless pericarp mutant allele, P^{wlw}_{m-1}, on mutation of a standard medium variegated pericarp allele in P^{wlw}_{m-1} P^{vv} heterozygotes (right) as compared with P^{wr} P^{vv} heterozygotes (left).

though a quantitative comparison was not made in this stock, it is evident that the amount of stabilization of P^{vv} induced by P^{vw}_{m-1} is of the same order of magnitude as that observed in light variegateds (cf. Wood and Brink 1956).

The ears in six families from $P^{vr}P^{vw}_{m-1} \times P^{vv}P^{vv}$ matings were divided according to cob color and then scored for variegation grade by matching with a standard set of P^{vv} ears, in which class 1 represented the least, and class 4 the largest degree of pigmentation. The results are summarized in Table 2.

TABLE 2

Grade of pericarp variegation in $P^{ww}_{m-1}\ P^{vv}$ *and* $P^{wr}\ P^{vv}$ *individuals from* $P^{ww}_{m-1}\ P^{wr} \times P^{vv}\ P^{vv}$ *matings*

Family	Parents	Cob color	No. individuals in variegation class				Av. variegation grade
			1	2	3	4	
63–159	Inbred W22 × 63–7–10	red	1	34	17	6	2.48
63–160	Inbred W22 × 63–7–10	red	1	22	28	5	2.66
63–163	63–27–4 × 63–7–10	red	0	14	12	2	2.57
	63–27–4 × 63–7–10	var.	1	38	4	0	2.07
63–164	63–27–7 × 63–7–10	red	2	17	20	0	2.46
	63–27–7 × 63–7–10	var.	3	27	2	0	1.97
63–333	63–27–5 × 63–7–6	red	1	29	5	0	2.11
	63–27–5 × 63–7–6	var.	1	33	0	0	1.97
63–335	63–27–8 × 63–7–6	red	7	27	12	0	2.11
	63–27–8 × 63–7–6	var.	3	40	0	0	1.93
63–336	63–27–11 × 63–7–6	red	1	29	14	0	2.30
	63–27–11 × 63–7–6	var.	6	34	2	0	1.90
63–340	63–27–16 × 63–7–6	red	3	15	10	3	2.42
	63–27–16 × 63–7–6	var.	1	31	2	0	2.03

Averages: red cob — 2.33
variegated cob — 1.98

The first two entries in the Table (families 63–159 and 63–160) represent control matings in which pollen from $P^{vv}P^{vv}$ plant 63–7–10 was applied to the original W22 P^{vr} inbred line itself. All these individuals, therefore, are of the $P^{vv}P^{vr}$ genotype. The two following families, 63–163 and 63–164, had the same pollen parent, but the pistillate parents were $P^{vw}_{m-1}P^{vr}$ individuals of W22 type. A sib $P^{vv}P^{vv}$ plant, 63–7–6, was used as the pollen parent on the inbred $P^{vw}_{m-1}P^{vr}$ individuals in the remaining families.

The scores for average variegation grade show that for all six segregating families fewer somatic mutations of P^{vv} to P^{rr} occurred in the $P^{vw}_{m-1}P^{vv}$ individuals than in their $P^{vv}P^{vr}$ sibs. The mean variegation grades of the two classes of ears are 1.98 and 2.33, respectively. These data show that the P^{vw}_{m-1} mutant regularly increases the stability of P^{vv} in heterozygotes. The similarity of the effect to that of two P^{vv} alleles on each other in $P^{vv}P^{vv}$ plants as previously analyzed by Wood and

BRINK (1956), suggests that it is Modulator action which is involved, and that *Mp* has the same potency in this respect in the $P^{wwo}_{m\text{-}1}$ mutant as in P^{vv}.

DISCUSSION

The simplest view which accords with the experimental facts presented is that $P^{wwo}_{m\text{-}1}$ differs from the variegated pericarp allele from which it arose only in the physical association of Modulator and the P^{rr} gene. Both alleles are combinations of *Mp* and P^{rr}, and it may be assumed that the large difference in stability between them is a reflection of the firmness with which Modulator is bonded at the *P* locus in the two cases.

The Modulator components of $P^{wwo}_{m\text{-}1}$ and P^{vv} were found to correspond closely in three respects. Both fully suppress the pigment-producing action of the P^{rr} gene, and both incite the same pattern of *Ds* chromosome breakage. Likewise, in $P^{wwo}_{m\text{-}1}P^{vv}$ heterozygotes, $P^{wwo}_{m\text{-}1}$ markedly depresses the frequency of somatic mutation of the P^{vv} homologue to P^{rr}, just as P^{vv} has been observed to do, reciprocally, in $P^{vv}P^{vv}$ homozygotes (WOOD and BRINK 1956).

$P^{wwo}_{m\text{-}1}$ and P^{vv}, on the other hand, are quite unlike in stability. No mutations to self-color were observed among 4742 $P^{wwo}_{m\text{-}1}$ gametes tested. It is estimated from unpublished data (BRAWN 1956) that an equal number of germ cells from medium variegated pericarp plants of similar breeding would have included about 90 such mutants. The single germinal mutation observed in the $P^{wwo}_{m\text{-}1}$ stocks involved a change from colorless, not to self-red, but to a fine grade of variegation.

Estimates of stability in somatic tissue likewise disclose a wide difference between $P^{wwo}_{m\text{-}1}$ and P^{vv}. Medium variegated ears ($P^{vv}P^{wr}$) in the W22 inbred line, carrying about 250 kernels each, average approximately five mutations of P^{vv} to P^{rr} per ear exceeding one eighth of the kernel circumference, the minimum size conveniently recorded. Among the 4330 heterozygous $P^{wwo}_{m\text{-}1}$ ears examined, 4229 showed no mutations to P^{rr}. The remaining ear, as mentioned earlier, showed only very fine red stripes, and probably represented a germinal mutation in the preceding generation of $P^{wwo}_{m\text{-}1}$ to an uncommon form of variegated pericarp.

A well recognized peculiarity of Modulator as a genetic unit is the capacity to undergo transposition from one location to another in the genome. Apparently the element can occupy a wide variety of sites (BRINK and NILAN 1952; BRINK 1954; N. W. VAN SCHAIK 1957). The present study shows that the firmness with which the element is held at or near a given locus varies greatly also. What this latter variation means in terms of physical relation of *Mp* to the genonema is quite unknown. It is significant that the stability in position of Modulator in the case of $P^{wwo}_{m\text{-}1}$ gives the latter the semblance of a stable allele. In fact, were it not for the fact that specia ltests, such as capacity to promote *Ds* chromosome breakage, for the presence of *Mp* at the *P* locus are available, $P^{wwo}_{m\text{-}1}$ ordinarily would be indistinguishable from the widely occurring stable P^{wwo} gene for colorless pericarp and cob.

The part which factors of the Modulator type play in chromosome organization is the central question on which this study bears. The concept that the genonema comprises a single category of severally specific determinants, physiologically independent of each other at this level and constituting the whole of the germinal substance, was brought into question by the cytological and genetic observations on heterochromatin in Drosophila (reviews by LEWIS 1950; HANNAH 1951). MCCLINTOCK has shown conclusively that a distinctive class of chromosomal elements occurs in maize which, by virtue of some kind of interrelationship with the conventional genes, likewise has pervasive effects on the genotype. These novel factors are pictured by MCCLINTOCK (1956) as specific controllers of gene action. Controllers are thought to fall into groups, the members of each group functioning as a system in modulating gene action within the nucleus. This view of the germinal substance calls for primary genetic variations of two classes, controller mutations and gene mutations. Gene expression in a given case is considered to be a function of the associated controller system. Furthermore, MCCLINTOCK assumes that the type of control over gene action exerted by the accessory elements conforms to the orderly requirements for cellular and tissue differentiation. It is implied also that mutation involving controllers is basic in regulating development of the individual.

The provisional nature of MCCLINTOCK's conclusions is evident when it is considered that none of the recent data on mutable alleles in maize excludes a simpler, albeit less well defined interpretation which has been associated with the concept of heterochromatin, particularly in Drosophila, since it was shown that this substance is not genetically inert as was first supposed. According to this view, which has been adverted to by various investigators (cf. HANNAH 1951) the heterochromatin is relatively homogeneous and serves some kind of residual genetic function essential to nuclear metabolism. Adequate criteria for regularly distinguishing heterochromatin from euchromatin have not been established, and there is a dearth of evidence concerning action of the substance. Consequently the problems in this area usually have been treated in pragmatic fashion, pending discovery of the facts whereby they may be more clearly defined and attacked.

The suggestion that heterochromatin embodies genetic elements having a generalized nuclear function has been given fairly explicit form by CASPERSSON (see CASPERSSON 1956, for a recent statement of his views). CASPERSSON postulates that the immediate products of a portion of the chromatin are massive amounts of unspecialized proteins and possibly other substances, which are not directly functional in differentiation but constitute a pool, so to speak, which is drawn upon in the formation of developmentally active substances. The latter comprise enzymes, constituted in part of material from the pool, but whose specificities, presumably, are attributable to genes each of which forms a limited quantity of a unique substance. Heterochromatin is suspected as the site of the determinants conditioning production of the substrate materials entering the nuclear pool, by way of the nucleolus or otherwise. This chromosomal component is considered to

be adapted to rapid production of large amounts of the required ground substances.

The assumed differentiation of the genetic material into what may be termed "kinetoplasm," comprising determinants of generalized function and genes in the conventional sense, is not at variance with any of the data on mutable alleles in plants or variegating position effects and heterochromatin in Drosophila. Such a postulate, on the other hand, does little more than connect in a provisional way facts which are still too isolated to permit of any firm interpretation.

If the chromosome includes kinetoplasm as a primary constituent with whose mass products genes with highly definitive effects interact to form the primary agents of morphogenesis, then mutable alleles may represent unconformities between the two classes of determinants. Various kinds of disarrangements of these factors may be visualized. One might picture Modulator, for example, as a "kinetomere," or unit of kinetoplasm, normal to the maize genotype in some particular, although unknown relation in most genotypes, but as adventive to the *P* locus quite by accident in the case of variegated pericarp. Conceivably the inhibiting effect of *Mp* at the *P* locus on pigment production could be brought about by (1) distorting the molecular configuration of the P^{rr} gene itself, (2) sequestering the immediate product of P^{rr} through formation of a physiologically inactive compound, (3) interference with fibrogenesis, coiling, or other processes whereby this region of the chromosome becomes functionally differentiated. A certain versatility in the character of the physical associations between *Mp* and P^{rr} is indicated by the fact that *Mp* is readily transposable in the case of ordinary variegated pericarp but fixed at the *P* locus in the stable colorless allele, $P^{wlw}_{m\text{-}1}$, here discussed.

McCLINTOCK (1951, 1956) has shown and others have confirmed the facts, that instability at a locus is a function not of the resident gene but of accessory chromosome material, and also that any locus, presumably, may become unstable, and in diverse ways. The evidence points definitively to primary chromosome elements as causal factors, essentially different from the conventional genes, and complementary to the latter in some, as yet, unknown way. But the data currently available are inadequate to establish where, in the hierarchy of chromosome organization, these components fit. The evidence can be construed as readily in terms of a kinetoplasm, comprising nuclear substrate factors, as of highly ordered controllers of gene action. These two views are antithetical, and the fact that either is tenable is witness to the indecisiveness of the evidence which suggests them.

SUMMARY

Colorless pericarp and cob is an infrequent mutation from the highly unstable variegated pericarp and cob allele, P^{vv}, in maize. The particular mutant here discussed, $P^{wlw}_{m\text{-}1}$, originated as a 43-kernel patch on a medium variegated ear. It is normally transmitted through the pollen and gives a plant of normal vigor when homozygous. Evidently, there, $P^{wlw}_{m\text{-}1}$ does not represent a deficiency at the *P*

locus. Examination of 4536 plants carrying $P^{vw}_{m\text{-}1}$ disclosed only one mutant. This individual bore an ear with very fine, red stripes not typical of any of the commonly encountered forms of variegated pericarp. Modulator was shown to be a regular component of the $P^{vw}_{m\text{-}1}$ allele. It could not be distinguished from that characteristic of the variegated pericarp stock from which it was derived in terms of the pattern of *Ds* chromosome breakage incited and reduction in frequency of P^{vv} to P^{rr} mutations in $P^{vv}P^{vw}_{m\text{-}1}$ and $P^{vv}P^{vv}$ plants. The evidence thus suggests that *Mp* is qualitatively the same in the $P^{vw}_{m\text{-}1}$ and P^{vv} alleles, and that the latter differ from each other essentially in the strength of bonding of *Mp* at the *P* locus. The indecisiveness of the evidence concerning the role in chromosome organization of elements of the Modulator type is briefly discussed.

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THE FREQUENCY OF LETHALS IN CROSSOVER AND NONCROSSOVER SECOND CHROMOSOMES OF DROSOPHILA MELANOGASTER*

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IT has long been known in different species that some individuals carry lethals even though they were descended from lethal-free ancestors. These lethals usually have originated by mutation at various loci. There exists, however, a second possible source for new lethals. Since individuals in a population are heterozygous for a large array of loci, recombination, it has been argued, might give rise to ill-adapted assortments of pre-existing alleles some of which might give lethal effects.

Evidence for the production of such "synthetic" lethals has been presented by WRIGHT and DOBZHANSKY (1946); MISRO (1949); and WALLACE, KING, MADDEN, KAUFMANN and MCGUNNIGLE (1953). The extensive work of the last named group of investigators concerned recombination among second chromosomes of *Drosophila melanogaster*. They found a considerable number of lethals among the offspring of females which had been made heterozygous for different lethal-free second chromosomes, in contrast to a small number of lethals from corresponding heterozygous males. In the females the presence of crossing over was considered to have been responsible for the origin of synthetic lethals while the absence of crossing over in the males did not lead to their occurrence.

Recent work by HILDRETH (1955, 1956) on the X chromosome and on the third chromosome of *Drosophila melanogaster* shows none of the marked deviations in the frequency of lethals from recombinant as compared to that from nonrecombinant chromosomes. Further, HILDRETH was able to localize at single loci the lethals which did occur in recombinant X and third chromosomes indicating that they were not due to lethal complexes synthesized by recombination.

In none of the former studies had there been a direct check on crossing over. The present experiment was so designed that specific crossover and noncrossover chromosomes could be selected and then tested for the presence of lethals. All data refer to the second chromosome of *D. melanogaster*.

PROBLEM AND METHODS

This test duplicated, in part, the techniques used by WALLACE *et al.* (1953). In each of seven "wild type" populations of *D. melanogaster* a single second chromosome was selected for the test. Flies that were homozygous for these "selected"

* This work paralleled other studies in this laboratory carried out with the support of the U.S. Atomic Energy Commission.

second chromosomes from each population were mated with flies from a single common population. Nonrecombinant and recombinant chromosomes were recovered from the interpopulational hybrids. Zygotes that were homozygous for these chromosomes, were tested for the presence of lethals.

Six of the "wild type" stocks used in this work were populations of diverse origin and histories which had been kept in the laboratory for long periods. Brief descriptions of these stocks are as follows: Stock 1. *Corona*, originally from Riverside, California; 2. *Formosa*, originally from Formosa, February 1950; 3. *Salta*, collected in Argentina, February 1950; 5. *Canton*, from the California Institute of Technology at Pasadena, 1939, originally 1A Canton S; 6. *Florida*, collected in Columbia, Florida. These stocks had all been kept by mass matings in the Genetics and Zoology Departments of the University of California at Berkeley. Stock 4. *Samarkand* was kept by brother-sister matings for 217 generations until June, 1953 and then by mass matings. The seventh stock, 7. *Napa*, was collected September 12, 1954 and kept by mass matings until the beginning of the test. This stock was established so that some tests could be made on a population not adapted to laboratory conditions.

The presence of crossovers in the interpopulational hybrids and the subsequent recovery of recombinant chromosomes were checked through the use of a second chromosome containing mutant genes. This "marked" chromosome was introduced into the test from the "common" population with which the other populations were hybridized. This chromosome contained the recessive mutants aristaless (*al* 2–0.0), black (*b* 2–48.5), cinnabar(*cn* 2–57.5), curved (*c* 2–75.5), and brown (*bw* 2–104.5). To a certain degree these mutants permitted the selection of nonrecombinant and recombinant chromosomes during the test.

Limitations to the recognition of recombination are based on the fact that the five mutant markers are not sufficient to exclude the occurrence of undetectable types of multiple crossovers. Groups of chromosomes in which all markers had remained together while primarily consisting of nonrecombinants would also contain some double crossover strands in which the two crossovers had occurred between the adjacent markers. Even occasional quadruple crossovers consisting of two doubles, each between adjacent markers, might have been present among the "nonrecombinants." Similarly, recombinant chromosomes, such as + *b cn c bw*, while usually the products of a single exchange between the loci of *al* and *b* might occasionally be the products of triple or even higher multiple exchanges which were so located as to leave the sequence *b cn c bw* unchanged. These reservations should be kept in mind when in the following pages the terms noncrossover, single crossover and, multiple crossover classes are used. These terms apply to the *recognizable* events only.

As a first step six separate lines were established from each of the seven wild type stocks. One second chromosome in each line contained the mutants, Curly (*Cy*) and Lobe (L^4), and an inversion in each arm as well as the recessive genes al^2, lt^3, and sp^2. The genotype of this chromosome, designated in this paper as *Cy al L*, is actually *Cy* al^2 lt^3 L^4 sp^2 with inversions *In*(2L)*Cy* al^2, *In*(2R) *Cy* lt^3

L^4 sp^2. The other second chromosome in each line was the descendant of a single second chromosome from the original wild type population. This chromosome was isolated by mating wild type flies with *Cy al L* flies and remating one +/ *Cy al L* male offspring with *Cy al L* females.

The *Cy al L* chromosome used in the crosses to isolate the single wild type second chromosome also served to keep that chromosome intact—inhibiting crossing over until flies were used in the final stages of the test for recombinational lethals.

Among the six lines from each stock one was selected for the main experiment. This selection was made: (1) upon the basis of the absence of inversions as indicated by salivary smears and (2) upon the absence of lethals in the isolated chromosome at this point in the crosses. The tests for prerecombinational lethals were made by mating *Cy al L* females with +/ *Cy al L* males from each line in each stock. The +/ *Cy al L* offspring were then mated. The development of wild type adults among the offspring of this last cross indicated the absence of a lethal while the presence of a lethal would lead to the appearance of Curly Lobe individuals only.

These tests for prerecombinational lethals at this stage served also as tests for the viability of the various nonlethal second chromosomes when in homozygous constitution. Taking the viability of the Curly Lobe class as unity the viability of the wild type homozygotes could be estimated by the deviation of the +/ *Cy al L* to +/+ ratio from the theoretical 2:1 ratio (Table 1).

While these selected wild type chromosomes assuredly were initially free of lethals the possibility existed that mutational lethals might arise in some of them. In order to distinguish such later prerecombinational lethals from lethals originating during the essential part of the experiment a special test was made. From each line, ten females heterozygous for a wild type chromosome and *al b cn c bw* were obtained and used separately to produce the next generation from which recombinant and nonrecombinant chromosomes were to be taken. If, as turned out to be the case in the crosses with chromosomes from the Canton population,

TABLE 1

Records of viability tests on second chromosome lines—F_1 of Cy al L/+ × Cy al L/+

Populations	Totals of flies produced in test of each line and percent of wild type flies in each test											
	A		B		C		D		E		F	
	Total	%	Total	%	Total	%	Total	%	Total	%	Total	%
1. Corona	263	23	283	19	40	17	219	27*	154	26	244	25
2. Formosa	402	24	256	0†	229	18	156	0†	115	20	285	39*
3. Salta	140	34*	81	19	135	40	157	42	138	23	82	0†
4. Samarkand	119	31	94	24	157	33*	122	27	116	26	48	6
5. Canton	111	29	198	29	68	25	115	21	77	32*	81	23
6. Florida	138	31*	25	20	173	27	109	25	34	8	99	23
7. Napa	93	22	134	28	173	27	303	34*	150	28	240	32

* Used in subsequent tests for recombinational lethals.
† Absence of wild type indicates presence of a lethal.

several lethals appeared at the end of the succeeding crosses and they could all be traced back to one heterozygous female, it was assumed that the lethal had been present in this female and could not be attributed to recombination. Such prerecombinational lethals were not included in the totals used in the analysis of the results of the tests.

The details of the mating scheme may be followed in Figure 1. Males homozygous for originally lethal-free, selected second chromosomes were mated with *al b cn c bw* females. F_1 females, heterozygous wild type (+/*al b cn c bw*), were mated with males from a specially prepared stock made up from the *al b cn c bw* laboratory line. In this stock the factors Star and asteroid (*S* 2–1.3 *ast* 2–1.34) were inserted into the marked second chromosome. This stock (*al S ast b cn c bw*/*Cy al L*) permitted the selection of offspring with nonrecombinant and recombinant chromosomes in the next generation and the factors Star and asteroid were used at a later point in the crosses as a necessary tool for classification (see F_3 offspring below). The F_2 generation contained flies with noncrossover and crossover second chromosomes over the marked chromosome *al S ast b cn c bw*. Fifty F_2 males of the noncrossover class (+), 50 males of each of the single crossover classes (*al*, *b cn c bw*, *al b*, *cn c bw*, *al b cn*, *c bw*, *al b cn c*, *bw*) and, a total of 50 males from various multiple crossover classes (usually seven each from double crossovers *al bw*, *b cn c*, *al b bw*, *cn c*, *al c bw*, *b cn* and two to three from triple crossovers *al c*, *al cn c*, *b cn bw*) were selected and individually mated with *al b c sp*/*Cy al*^{2} *lt*^{3} *L*^{4} *sp*^{2} females. Thus a total of 500 males from each of the seven test series were selected from the F_2 offspring and mated in individual "creamers" (one ounce glass containers).

It was not possible to maintain a constant number of tests for each population —or constant number of tests for each wild type or crossover class—tested for recombinant lethals. During the course of the tests with each population a few of the cultures did not produce any flies, or not enough flies in some cases. In the experiments with the Samarkand population the bottles became heavily infected with mold and considerable numbers of the cultures did not develop. The tests using the Napa population had to be repeated because excessive heat which occurred during the time of the F_2 cross rendered a great number of the test cultures sterile.

The Curly Lobe F_3 offspring in each test consisted of two types, namely flies with (1) a wild type, noncrossover, chromosome or with one of the various recombinant chromosomes respectively, and (2) flies with the *al S ast b cn c bw* chromosome. Flies of group (1) were selected for the next mating; those of group (2) which showed the dominant mutant Star were discarded. The selected F_3 flies were mated among themeselves, several males to several virgin females. In the next and last generation, zygotes homozygous for the wild type or the various recombinant chromosomes were produced. Provided no lethal was present and assuming full viability, the expected ratio of Curly Lobe heterozygotes to wild type, or recombinant, homozygotes was two to one. The absence in any of these F_3 cultures of wild type or recombinant offspring indicated a lethal. Curly Lobe

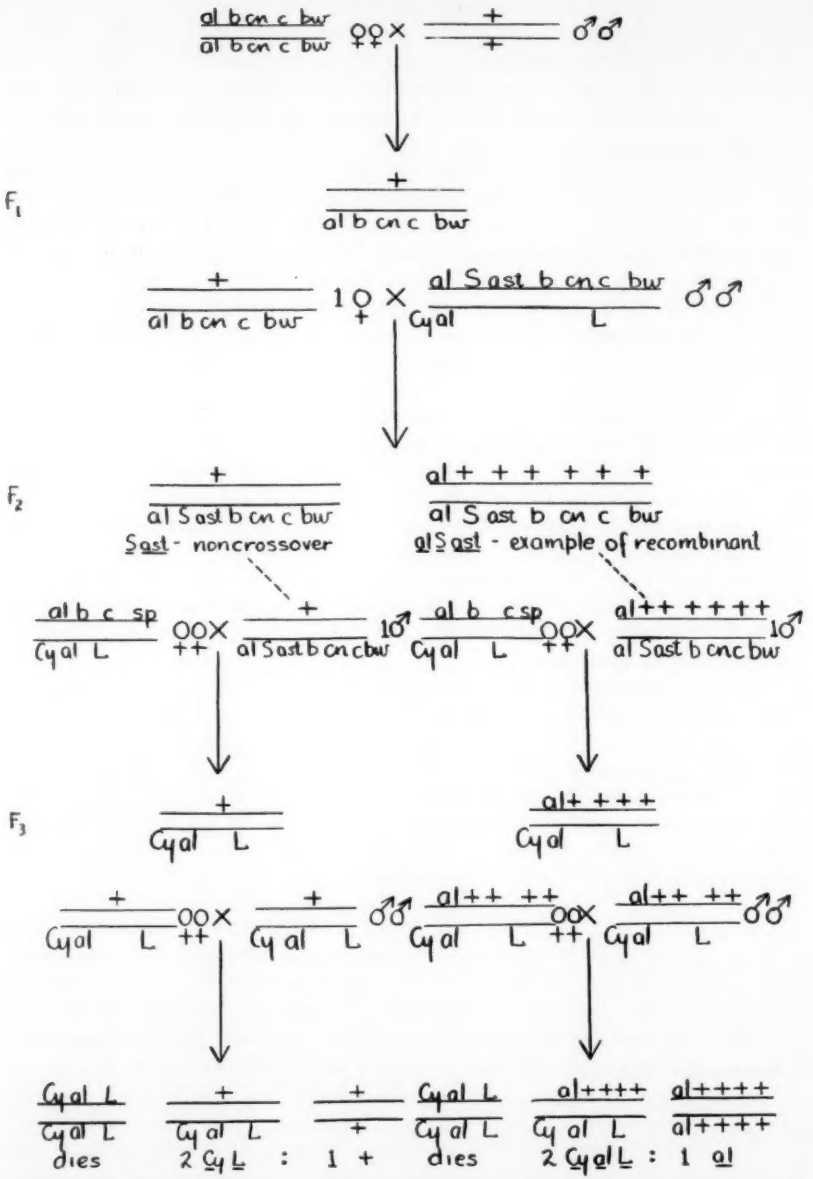

FIGURE 1.—Mating scheme for test for recombinational lethals in the second chromosome of *D. melanogaster*. The mating scheme presented here shows the test with the nonrecombinant chromosome and one example, only, with a recombinant chromosome.

heterozygotes from tests indicating the presence of lethals were remated and a final check for lethals was made in the succeeding generation.

At the conclusion of these tests it was felt that the significance of the results was limited by the small number of "control", or noncrossover, chromosomes that had been included. A series of crosses generally comparable to those used in the foregoing tests were designed and carried out to test the frequency of lethals in additional noncrossover chromosomes. In these crosses the noncrossover chromosomes came from males and thus were not only phenotypically noncrossovers but also did not include undetectable multiple crossovers. Males from six of the seven wild type lines that had been selected earlier were mated with *al b cn c bw* females. The +/ *al b cn c bw* male offspring were mated with *Cy al L* virgin females. At the next step a viability test was introduced into the procedure and at the same time the test for lethals in the nonrecombinant chromosomes was begun for each of the wild type chromosomes by mating approximately 100 +/ *Cy al L* males from the preceding cross with virgin females containing the "marked" second chromosome carrying the mutants Star and asteroid. The viability test was similar to the one performed in the first series of tests where *Cy al L* females were mated with +/ *Cy al L* males, the +/ *Cy al L* offspring were mated and the subsequent development of wild type offspring indicated the absence of a lethal. If this viability test indicated the absence of lethals in the chromosome being tested then the final cross was made using males and virgin females of the genotype +/ *Cy al L* from the +/ *Cy al L* × *al S ast b cn c bw* / *Cy al L* mating. The absence of flies homozygous for the nonrecombinant wild type chromosome in the offspring indicated presence of a lethal. As in the preceding tests all suspected lethals were retested for another generation.

For practical considerations, and for simplicity in handling the lethal studies, it was decided that the measure of lethality would be the absence in the individual tests of any wild type flies or of flies of the various crossover classes. The experimental procedure consisted of placing etherized flies from each F_3 culture on a counting plate. With the microscope, the offspring were examined for the presence or absence of wild type or of recombinational flies. In both test series, the absence of wild type or of recombinational flies in fifty or more flies was considered evidence for the presence of a lethal factor. If individual bottles produced fewer than 50 flies they were recultured and counted for lethals at the next generation.

All tests were made at 25°C. All culture bottles contained cornmeal-agar-molasses medium enriched with brewer's yeast. The F_3 cultures were examined on the 15th day after mating. The ages of the F_1 and F_2 parents were kept within a variable period of four days for each group, i.e. F_1 and F_2 matings began at the 11th day after culturing and ceased at the 15th day in each case.

RESULTS

A total of 23 F_3 creamers from both series of crosses did not contain wild type or recombinational flies among the offspring, indicating the presence of lethals in the selected second chromosomes. Eighteen, or 78.3 percent of the suspected lethals were confirmed in the F_4. Of this total of 23 lethals, 18 occurred in test 1,

with the heterozygous females. Fourteen of these were confirmed in the F_4, i.e. they did not produce homozygous offspring. The remaining five lethals occurred in test 2, with heterozygous males, four were confirmed in the F_4, one was not confirmed.

No attempt was made to measure poor viability. Creamers in which any wild type flies, or flies of the various crossover types, occurred were counted as nonlethal.

The results of the tests are shown in Table 2 where the seven wild type chromosomes used are listed at the left. The frequencies of lethals recovered are represented in fractional form for each of the wild type and recombinant chromosomes used. Recombinant chromosomes from multiple crossover are listed only as "multiple." When a lethal did occur with recombinant chromosomes from multiple crossover, as in the tests with the Samarkand and Salta populations, the particular recombinant second chromosome involved is listed at the bottom of the table.

In one test with the Canton population, several lethals occurred but were traceable to the same F_1 heterozygous female. These are assumed to have been lethals present in that female and not the result of crossing over, i.e. prerecombinational lethals. They are not included in the totals and percentages presented at the right and at the bottom of the table.

After the elimination of the prerecombinational lethals from the Canton test results, the final totals and percentages do not indicate a significant increase over a reasonable expectation for a spontaneous mutation rate. In different sets of tests the frequency of lethals varies from one in 595 chromosomes, or 0.2 percent lethals, to four in 578 chromosomes, or 0.7 percent lethals. DUBININ (1946) gives the figure 0.53 percent lethals expected per generation for the second chromosome.

A comparison between two sets of observations—the frequency of lethals occurring in the controls, noncrossover or wild type flies, and the frequency of lethals occurring in the experimentals, recombinant flies—does not indicate a significant difference in lethals in recombinant flies. The frequency of lethals in the controls is five in 970 chromosomes tested (0.5 percent). The frequency of lethals in the recombinants is 13 in 3063 chromosomes tested (0.4 percent). A homogeneity test of these two sets of data yields a chi square indicating a probability greater than 0.7. The difference between the two lethal frequencies is, therefore, not statistically significant.

The comparison of frequencies of lethals in recombinant chromosomes recovered from apparent single and multiple crossovers in the second chromosome likewise gives a probability greater than 0.7 in favor of a chance deviation.

The comparison of frequency of lethals in recombinant chromosomes recovered from the test performed on the nonlaboratory stock (Napa) with the frequency of lethals in recombinant chromosomes recovered for tests on laboratory stocks shows a probability greater than 0.7 in favor of chance deviation.

It is not clear wherein the difference lies between the results of WALLACE *et al*,

TABLE 2

Results from the tests for recombinational lethals in the second chromosome of D. melanogaster

Origin of wild type chromosomes	Frequency of lethals in wild type and various recombinant chromosomes tested																
	Noncrossovers (+)				Crossovers												
	Females	Males	Total	%	*al*	*b cn c bw*	*al b*	*cn c bw*	*al b cn*	*c bw*	*al b cn c*	*bw*	Multiples	Total	%	Grand totals§	%
Corona	0/50	2/98	2/148	1.0	0/50	0/47	0/50	0/50	0/50	0/50	0/50	0/50	0/52	0/499	0	2/597	0.3
Samarkand	0/28	0/100	0/128	0	1/38	0/33	0/38	0/27	1/49	0/33	0/42	0/31	1/27†	3/346	0.8	3/446	0.7
Formosa	0/50	0/100	0/150	0.6	0/50	0/48	0/47	0/50	0/50	0/50	0/50	0/50	0/50	0/495	0	1/595	0.2
Salta	1/56	0/86	1/142	0.7	0/46	0/49	0/48	1/46	0/48	0/53	1/48	0/49	1/49‡	4/492	0.8	4/578	0.7
Napa[1]	0/43	....	0/200	0	0/23	0/10	0/3	0/5	0/13	0/16	0/8	1/11	0/22	(1/154	0.6)		
														2/627	0.3	2/739	0.3
Napa[2]	0/45	0/112			0/41	0/47	0/50	0/50	0/42	1/50	0/50	0/50	0/48	(1/473	0.2)		
Canton	0/50	....	0/50	0	0/47	0/45	1/50	0/47	0/50	1/46	0/46	0/49	0/57	2/487	0.4	2/487	0.4
					2/49	2/47	1/50	1/48	0/50	3/48	0/46	1/50	0/57	10/495*			
Florida	0/50	1/102	1/152	0.6	0/48	1/47	1/50	0/50	0/49	0/50	0/45	1/48	0/52	3/489	0.6	4/591	0.7
Totals	1/372	4/598	5/970	0.5	1/343	1/326	2/336	1/325	1/351	2/348	1/339	2/338	2/357	14/3435	0.4	18/4033	0.4

[1] Results 6-4-55.
[2] Results 10-5-55.
* Eight lethals in the Canton population test (*al* two; *b cn c bw* two; *cn c bw* one;*c bw* two of the three; *bw* one) occurred in the F_2 cultures from F_1 flies produced from the same F_1 female (no. 5). Presence of a prerecombinational lethal was assumed and these lethals are not included in the final totals or percentages.
† Recombinant chromosome contained the mutants *b cn*.
‡ Recombinant chromosome contained the mutants *b cn c bw*.
§ From noncrossovers and crossovers.

who reported the finding of large numbers of recombinational lethals and the present study which shows their absence. To some extent the selection of wild type second chromosomes with high viabilities may have reduced the chance of recombinational synthesis of lethals. It may also be argued that six of the wild type chromosomes as well as the marker chromosome came from long established laboratory stocks each of which may have become internally adapted to the laboratory environment. Even if this supposition is accepted one still would expect different coadapted genotypes in these different stocks and a subsequent creation of less adapted genotypes, including lethal ones, by recombination. It is likely that poorly adapted, but still viable, genotypes did indeed originate since the experiments were designed to discover lethal genotypes only. Possibly some of the lethals discovered by former workers would have been semilethals under our own culture conditions and therefore could not be included in the present tally of lethals.

SUMMARY

A test was made for lethals in recombinant second chromosomes of *D. melanogaster*, using techniques similar to those of other authors. In addition, the recovery of recombinant chromosomes was made certain through the use of a chromosome marked with five recessive mutant genes. This allowed for the specific observation of particular second chromosome loci involved in any recombinant chromosome containing a lethal. The experiments were so designed as to permit elimination of prerecombinational lethals from the test results. The wild type second chromosome from each of seven populations was selected for high viability before the test began.

The results indicate no statistical deviation between recombinant and nonrecombinant chromosomes in producing lethals. Furthermore, the frequencies of lethals among the various populations tested show no striking dissimilarities. It seems likely, then, that recombination has played no great part in the production of the lethals recovered in this test.

ACKNOWLEDGMENTS

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RADIATION DAMAGE IN THE DEVELOPING GERM CELLS OF DROSOPHILA VIRILIS FROM FAST NEUTRON TREATMENT[1]

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THE search for biological responses which might indicate differences in the mode of action of radiations with different ion densities has utilized various types of biological systems of the plant and animal kingdom (EHRENBERG 1954; SYMPOSIUM 1954). One characteristic of densely ionizing radiations such as fast neutrons and alpha particles is the small change in the amount of radiation damage in response to modifications of the physio-chemical systems of the cell (GILES *et al.* 1952; CONGER, cited in GILES *et al.* 1952; EHRENBERG and NYBOM 1954). With the less densely ionizing radiations of X-rays, such modifications as water content, oxygen concentration and temperature changes become a predominant factor for changing the total amount of radiation damage induced in biological systems. (Gray *et al.* 1953; EHRENBERG *et al.* 1953; THODAY and READ 1947).

Previous studies with the densely ionizing radiations such as alpha particles and neutrons have been comparisons of damage in one type of cell under several physiological conditions (GILES *et al.* 1952; GRAY *et al.* 1953) or direct comparisons of damage in germ cells of different types in the males and females (KING and WOOD 1955). These studies have not included comparative tests of biological damage in a series of different types of cells which were exposed to irradiation treatment at the same time. By using the immature germ cells of *Drosophila virilis* a direct comparison of the amount and type of radiation damage can be made in postmeiotic sperm and spermatid cells, in meiotic cells (spermatocytes) and in premeiotic spermatogonial cells. The present investigation reports the resulting biological damage measured as translocations and dominant lethals from fast neutron treatment in such a series of immature germ cells of spermatogenesis.

MATERIALS AND METHODS

Fast neutrons were obtained from an uranium (U^{235}) converter plate in the thermal column of the Brookhaven reactor. The neutrons have an energy distribution which is the same as the fission spectrum of U^{235} and the total neutron flux

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in the thermal column is composed of 55 percent neutrons with energies of 0.1 to 1.5 Mev, 38 percent with energies of 1.5 to 2.5 Mev, and 7.0 percent with energies of 2.5 to 6 Mev. The detailed dosimetry for the neutron facility has been reported by HANDLOSER and DELIHAS (1955). A neutron flux of 3.5 to 4.0 $\times$ 10^8 n^f/cm^2/sec and gamma irradiation at a maximum rate of 420r/hr have been calculated for the position of the biological material (CURTIS *et al.* 1956; DELIHAS private communication).

Treatment times of 2½, 5 and 10 minutes in the thermal column were used. The fast neutron doses would be 600, 1200 and 2400 $\times$ 10^8 n^f/cm^2/sec for tests 2A, 2B and 2C. The gamma rays would contribute doses of 18, 35 and 70r for the three treatment periods. The maximum correction values calculated from cobalt-60 data (ALEXANDER, unpublished) would account for such a small percentage of the total biological damage that any differences in the radiation damage can be attributed primarily to neutron radiation.

The biological tests are similar to those used in previous tests with X-radiation (ALEXANDER and STONE 1955). Samples of young males of *Drosophila virilis* (Texmelucan, Strain 1801.1) were collected one to three hours after eclosion and treated with fast neutrons at 18–21 hours of age. Mass matings were used for the first five days after treatment since *virilis* males do not have mature viable sperm for six or seven days after emergence. After this maturing period, the treated males were remated, individually, to three females—one Texmelucan normal and two carrying the markers broken, tiny bristle, gapped, cardinal and peach. The males were remated to virgin females every two days over a period of 18 days. Germ cells which were treated in various developmental stages of spermatogenesis were sampled by this remating procedure. The cells which were more mature at the time of irradiation were sampled first (mating period A) and younger, immature cells are represented by the following mating periods (B, C, D).

Samples for the dominant lethal egg count tests were obtained from the Texmelucan (normal) females after separation from the treated males. Egg counts were made from individual females for each of the nine mating periods (A through I). The percentages for dominant lethals were calculated by determining the ratio of the number of pupae produced from the total sample of eggs. The Texmelucan females were also used to obtain egg samples for the sperm fertilization tests. These tests were used to separate eggs that were fertilized but failed to develop from eggs that did not develop because of absence of sperm. Samples of eggs were collected within one hour after being deposited by the females and checked microscopically for the presence of sperm. Although only a limited number of mating periods could be sampled, these periods included germ cells treated in the postmeiotic, meiotic and premeiotic stages to detect any differences in lethal types.

For the different mating periods translocations were measured by genetic tests using crosses between treated males and marker females containing the recessive mutants, broken (*b*, chromosome 2), tiny bristle, gapped (*tb*, *gp* on 3), cardinal

(*cd* on 4), and peach (*pe* on 5). The X and sixth chromosomes were not marked, but translocations involving the Y can be detected by measuring differences in the recombination classes in males and females. The F_1 males, heterozygous for the marker genes and treated chromosomes, were backcrossed individually to three marker females. Samples of ten F_1 males were used from each P_1 cross. The genetic recombination classes of the recessive markers among the F_2 progeny from each F_1 male indicated the presence or absence of translocations.

RESULTS

The detailed results of the translocation and dominant lethal tests are given in Table 1. The translocation damage recovered during each mating period is re-

TABLE 1

Dominant lethal and translocation damage in various types of immature germ cells of Drosophila virilis

Experiment	Period	Type and number of translocations				Total sperm tested	Percent sperm with translocation ± 2 SD	Dominant lethals			Eggs fertilized	
		T_2	T_3	T_{2+2}	T_4			Eggs	Pupae	Percent	Number with sperm	Number without sperm
Test 2A												
600 × 10^8 n^f/cm^2	A	7	2	.	.	306	2.9±1.9	356	283	20.5	..	..
	B	7	.	.	.	342	2.1±1.5	2810	2045	27.2	..	..
	C	14	.	.	.	354	3.9±2.0	1403	1167	16.8	..	..
	D	16	1	.	.	282	6.0±2.8	1252	852	31.9	..	..
	E	2	.	.	.	95	2.1±2.9	261	155	40.6	..	..
	F	.	.	.	.	47	0.0	718	612	14.9	59	4
	G	.	.	.	.	126	0.0				0	16
	H	.	.	.	.	108	0.0	1935	1703	12.0	..	..
	I	.	.	.	.	52	0.0	1173	974	17.0	26	3
Test 2B												
1200 × 10^8 n^f/cm^2	A	19	2	.	.	315	6.7±2.8	1635	947	42.1	..	..
	B	17	1	.	.	330	5.4±2.6	2862	1700	40.6	..	..
	C	26	1	.	.	339	8.0±3.0	1604	1036	35.4	18	5
	D	15	1	1	.	205	8.3±3.9	952	408	57.1	9	0
	E	1	.	.	.	100	1.0±0.0	95	50	47.4	21	1
	F	.	.	.	.	71	0.0	333	237	28.8	..	..
	G	.	.	.	.	95	0.0	...	...	...	..	..
	H	.	.	.	.	94	0.0	1150	952	17.2	60	3
	I	.	.	.	.	63	0.0	613	549	10.4	..	..
Test 2C												
2400 × 10^8 n^f/cm^2	A	No test										
	B	29	4	2	.	254	13.8±4.3	2527	1090	56.9	..	..
	C	27	.	.	.	166	16.3±5.5	1528	555	63.7	..	..
	D	13	2	1	1	90	18.9±7.8	266	55	79.3	..	..
	E	2	.	.	1	10	30.0±0.0	152	37	75.7	..	..
	F	.	.	.	.	7	0.0	491	219	55.4	47	2
	G	.	.	.	.	76	0.0	...	...	...	0	21
	H	.	.	.	.	55	0.0	595	395	33.6	30	9
	I	.	.	.	.	44	0.0	714	580	18.8	52	2

ported as the number of translocations recovered and the number of chromosomes involved (T_2 for two and T_3 for three chromosomes, etc.). In several cases at the highest dosage two different translocations were recovered from the same sperm. These were counted as due to a single hit since the nature of fast neutron radiation makes it probable that all breaks were produced from one hit. For the dominant lethal tests, the total egg sample and number of pupae produced are given with the resulting percentage of dominant lethals for each mating period. Sperm fertilization tests were used to separate dominant lethal effects into two categories (1) those in which eggs contained sperm but failed to develop and (2) those in which the eggs failed to develop due to the absence of sperm.

Translocations indicate that multiple chromosome breakage and recombination occurred in postmeiotic cells. The effect of the same neutron dose upon the different types of postmeiotic cells can be seen in test 2A (Table 1). More mature cells (A and B) gave translocation frequency values of 2.9 and 2.1 percent whereas in the younger cells (type C) this damage increased to 3.9 percent. The most sensitive postmeiotic cells were those from mating period D where 6.0 percent of the sperm contained translocations. At all three doses, a small consistent increase in the translocation damage is evident when the damage in immature cell types C and D is compared to that in the mature cells A and B.

In Figure 1, the percentage of translocations produced in germ cells from different mating periods are plotted for each neutron dose. The regression lines were determined by the formula, $y = bx + a$ where $b = \frac{\Sigma xy}{\Sigma x^2}$, x = the neutron dose, y = translocation damage and a = the control rate. The control value for spontaneous translocations in males is so small (see ALEXANDER and STONE 1955) that

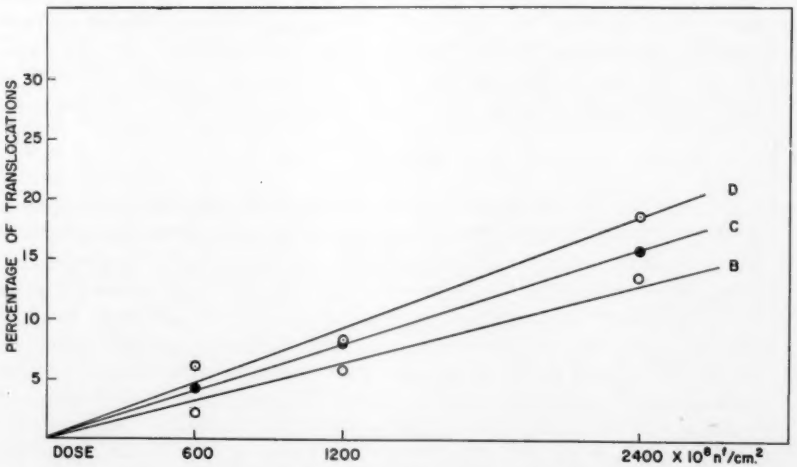

FIGURE 1.—Translocation damage in postmeiotic germ cells of *Drosophila virilis*.

a control correction is not included and the regression line is determined by bx. A comparison of the regression lines for postmeiotic cells which were sampled during the mating periods B, C and D show that there is a gradual increase in the sensitivity for chromosome breakage in spermatids. The relative sensitivity of the B and D type of cells can be expressed by the difference in the neutron dose to produce the same amount of damage. At the lowest dose 600×10^8 n^f/cm^2, a value of 6.0 percent translocations was obtained for cells of the D period; twice this dose is necessary to produce this much damage in the B type cells.

A comparison of the dominant lethal damage throughout the germ cell cycle shows that a greater percentage of lethal damage was recovered from all types of postmeiotic cells (B, C, D) than from meiotic (F) or premeiotic (H) cells. The dominant lethal values from samples obtained in mating period I (Table 1), with all three doses, were no greater than six percent above the control level and were not considered different from the control values. In Figure 2, the percentage of dominant lethal damage in each of the various types of cells is expressed by a straight line using the formula: $y = bx + a$ where $b = \frac{\Sigma xy}{\Sigma x^2}$; x is the neutron dose, y = the dominant lethal damage and a = the control value of 12 percent.

The relative amount of lethal damage from the various types of postmeiotic, meiotic and premeiotic germ cells can be determined by inspecting Figure 2. The postmeiotic B, C and D cells show a gradual increase in sensitivity to neutron treatment with the highest damage in D type cells. The highest translocation rate was also recovered from this type of cells. The relative sensitivities of the various types of germ cells in the cycle can be expressed by showing the amount of radiation necessary to produce equal amounts of damage (Table 2). The least sensitive cells of the cycle, H, were used as a standard. A comparison of the least sensitive premeiotic cells (H) and the most sensitive postmeiotic cells (D) shows that H cells will require a dosage almost three times that required by D cells to produce an equal amount of damage. Sensitivities for postmeiotic cells vary from

TABLE 2

Dominant lethal damage in the immature germ cells of Drosophila virilis

Type of cell	Dose (10^8 n^f/cm^2) for same percentage of dominant lethals	Relative sensitivity
Premeiotic		
H	2400	1.0
Meiotic		
F	1500	1.5
Postmeiotic		
B	1320	1.8
C	1300	1.8
D	900	2.7

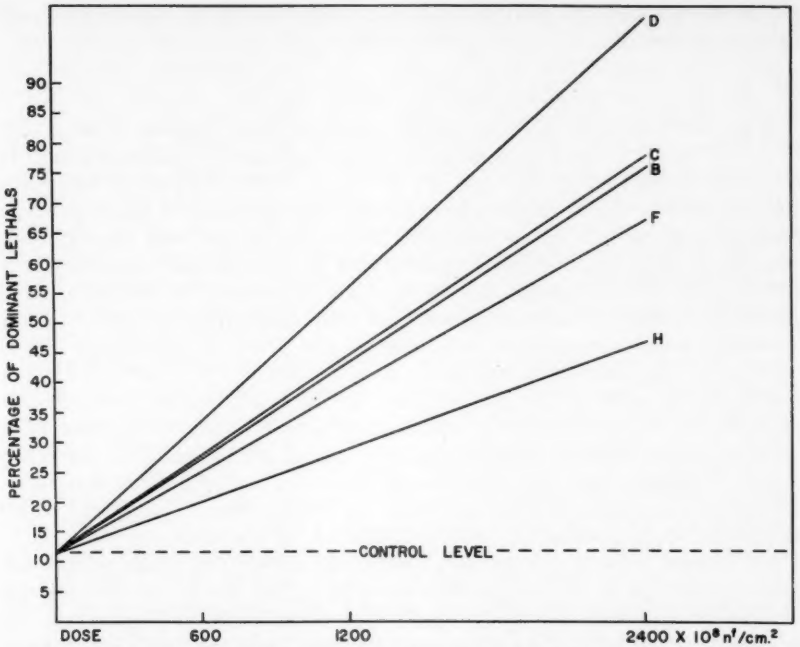

FIGURE 2.—Dominant lethal damage in the immature cells of *Drosophila virilis* fitted to a trend line ($y=bx+a$)

1.8 to 2.7 and a dosage difference of about two times is required to produce as much damage in the less sensitive B type as in D type cells.

The sperm fertilization tests for postmeiotic cells were checked in Test 2B. Most of the eggs contained sperm which shows that their failure to hatch (lethal) is not due to the absence of sperm. The radiation damage must be attributed to a genetic effect since the sperm fertilized the egg but development did not result. The meiotic cells from mating period F were checked at the lowest and highest dose, and neither showed a value different from the value of 90 to 95 percent for the percentage of eggs containing sperm in the control test with the Texmelucan stock. Treated males were mated to three marker females in tests for period G. Samples were difficult to obtain but the few eggs which were checked contained no sperm showing a marked reduction in the number of mature sperm in this period. The data differ from the control data since at least 25 of the 37 eggs would be expected to contain sperm. However, sperm can be obtained in mating period G since some F_1 offspring were produced in the translocation test. The tests from mating periods H and I showed no lack of sperm for egg fertilization. Mating period G is the only period checked in which "dominant lethals" resulted from

the absence of sperm for fertilization—thus indicating degeneration of germ cells in the treated males.

DISCUSSION

A variation in the amount of damage recovered from different types of cells in the germ cell cycle of spermatogenesis was reported for dominant lethals in *D. melanogaster* by LUNNING (1952). AUERBACH (1954) tested for autosomal and sex-linked recessive lethals and rearrangements in various types of pre- and postmeiotic germ cells in *melanogaster*. With X-radiation, both types of rearrangements, deletions and translocations, reached a maximum simultaneously with recessive lethal damage. Similar results were obtained for the induction of rearrangements in the various types of germ cells of the cycle in *D. virilis* and the amount of breakage was shown to be influenced by pretreating in gases such as nitrogen, oxygen and carbon monoxide (ALEXANDER, CLAYTON and STONE 1954). The results for radiation damage in the spermatogenic cycle in *melanogaster* and *virilis* have been established from repeated experiments by various workers. With densely ionizing radiation such as neutrons (above 200 ion pairs /μ) the direct action of ionizing particles upon the chromosomes is the predominant factor whereas with sparsely ionizing radiations like X-rays, chemical changes and cell physiology become important. With the radiations of lower ion densities such as X-ray (80 ion pairs /μ) the primary radicals, H and OH, are produced in a diffuse way and are at sufficient distances so that oxygen and chemical protectors can intervene in the chemical reactions. With a diffuse distribution of radicals the physiological activity of the cell becomes important for determining the type and amount of radiation damage to the cell. With the densely ionizing radiations, OH radicals can be formed in close approximation and reactions occur independent of the oxygen concentration or presence of other chemical compounds (ALLEN 1948). The general principles of biological effectiveness, ion density of radiations and the chemical basis for radiation damage are discussed elsewhere (see EHRENBERG and NYBOM 1954; BARQ and ALEXANDER 1955).

The data from the neutron treatment of the germ cell cycle of *virilis* show that the biological damage in any one type of cell increases proportional to an increase in dose. These tests agree with the "one-hit" phenomena from neutron bombardment which results from one proton track being sufficient to cause multiple chromosome breakage (see LEA 1946; CATCHESIDE 1948). Each of the various types of immature cells showed a damage curve which would be expected from this type of action. Mature sperm cells also showed a proportional increase in dose for fission neutrons (STONE *et al.* 1954). With fission neutrons from a reactor source and nuclear detonations, both translocation and lethal damage in each of the various types of germ cells increased proportional to an increase in dosage. Sensitivity differences to neutrons treatment for the different types of cells (Table 2) result in a series of damage curves for the cells (Figures 1 and 2).

The results for meiotic and premeiotic cells differed from those for postmeiotic cells in that translocations were not recovered from meiotic and premeiotic cells

and lower dominant lethal values were observed. The rarity of two break arrangements in cells treated in meiosis and absence in those treated in the premeiotic stages has been shown in the previous X-ray studies with *virilis* as well as with *melanogaster* (AUERBACH 1954; WARD and ALEXANDER 1957). The results from Drosophila females (GLASS 1955a, b), Sciara females (BOZEMAN and METZ 1949) and Habrobracon females (WHITING 1945a, b) were similar. The lower amount of dominant lethal damage in all meiotic and premeiotic cells must be due, in part, to a type of selective survival of breaks produced in meiotic and premeiotic germ cells. Such a densely ionizing radiation as neutrons would be expected to produce chromosome breakage in meiotic and premeiotic cells although a variation in the sensitivity of the chromosomes to breakage also appears to be necessary for a complete explanation for the lower amount of radiation damage recovered from these cells.

The dominant lethal damage which was recovered from meiotic and premeiotic cells showed a gradual drop in percentage as sampling proceeded into the younger meiotic and premeiotic cells (Periods F and H). By Period I, the lethal damage was not different from the control value at either of the three neutron doses. Spermatozoa sampled in this period 21-23 days after irradiation treatment were most probably treated as spermatogonial cells. A similar situation has been reported for mice after low doses of neutrons (RUSSELL, RUSSELL and KIMBALL 1954). In the mouse tests, spermatozoa obtained in matings 24-31 days after exposures to 22 and 50 rep doses of neutrons were almost free of dominant lethals. In the *virilis* tests the doses are too low to determine the nature of the effect of neutrons in spermatogonial cells although a fourfold difference in dose would be expected to produce a greater difference in dominant lethals than obtained in the tests.

The sperm fertilization tests with *virilis* show that neutrons produce cell degeneration in some spermatogonial types. The absence of spermatozoa in the males 17-19 days after irradiation is shown by the absence of sperm in the egg samples from females which were taken from that period and used for matings. The failure of egg development in this period is due, in part, to the absence of sperm for egg fertilization. Similar results have been reported with X-ray for *virilis* (ALEXANDER and STONE 1955) and *melanogaster* (AUERBACH 1954).

Both translocations and dominant lethals were recovered from the postmeiotic germ cells. The dominant lethals are presumed to result from damage to the genetic system since no degeneration of germinal cells treated after meiosis has been detected. Fast neutron radiations with ion densities of 1200 ion pairs/μ (DELIHAS, private communication) produce small consistent differences in the amount of biological damage in this series of nondividing germ cells. CLAYTON (1957) has found by histological examination that all stages of spermiogenesis as well as nonmotile sperm are represented in this sample. The high ion density suggests a direct effect upon the chromosomes which vary as to sensitivity for chromosome breakage. The results obtained by EHRENBERG and NYBOM (1954) and EHRENBERG *et al.* (1953) with barley seed indicate a direct action of neu-

trons. In these tests more radiation damage was produced in germinating barley seed than dry ones; however, there was no difference in the radiation damage in germinating seed whether the irradiations were carried out in vacuum, air or oxygen.

In previous tests with *virilis* using radiations of lower ion density, i.e. X-ray (80 ion pairs/μ), physiological changes such as oxygen concentration and temperature result in a greater degree of reduction or enhancement of radiation damage in the young spermatid cells than in nonmotile sperm. SOBELS (1955) found a similar effect in the immature cells of the *melanogaster* cycle when he combined pretreatments of catalase inhibiting substances such as azide and cyanide and X-radiation. Experiments with *virilis* are in progress to test the effectiveness of oxygen to influence radiation damage in the spermatogenic cycle with neutron irradiation.

When the relative efficiency of fission neutrons and 200 Kv X-rays are compared in the immature germ cells, there is a variation in the values obtained for the various types of cells. By using: Relative Biological Efficiency (RBE) = $\frac{\text{Dose for 50 percent dominant lethals-200 Kv X-rays (RADS)}}{\text{Dose for 50 percent dominant lethals-fission neutrons (RADS)}}$ and calculating rad units (relative absorbed dose) for fission neutrons using Handbook 63 (1957) the following dose relationships can be compared for the various types of cells. For sperm, 2880/432 = 6.6; spermatids (period C) = 1250/410 = 3.1; spermatids (period D) = 750/233 = 3.2; spermatids (period E) = 570/250 = 2.3 and meiotic cells (period F) = 790/487 = 1.6. The value for the relative effectiveness of neutrons is larger in mature sperm than in spermatids. These variations are produced by differences in the relative sensitivity of sperm and spermatids with neutron and X-rays. With neutrons about one half the dose is necessary to produce the same amount of damage in spermatids as in mature sperm and with x-radiation only one fourth the dose is required. The higher sensitivity of spermatids to x-radiations corresponds to the period of influence of environmental changes upon radiation damage. The influence of environmental changes with neutrons and X-rays would allow more damage in mature sperm with neutrons and greater increases in damage with X-rays in spermatids to produce lower RBE values. Similar amounts of lethal damage were obtained for meiotic cells with neutrons and X-ray.

SUMMARY

The radiation damage produced by the densely ionizing radiations from fission neutrons was tested by studies of various stages of post- and premeiotic germ cells of *Drosophila virilis*. Young males were treated with doses of 600, 1200, and 2400 $\times 10^8$ n^f/cm^2 and the various types of cells sampled by successive remating periods. The induction of translocations and dominant lethals in the various stages of spermatogenesis was tested.

Translocation and dominant lethal damage produced from neutron treatment in the immature germ cells increased proportionally with an increase in dose. The

damage obtained for each type of cell in the spermatogenic cycle resulted in a curve which would be expected when multiple chromosome breakage is produced from a single proton hit. The radiation damage increased linearly with dose in the various types of cells although the relative sensitivity of the various stages was not the same.

Postmeiotic germ cells (B-D) varied in sensitivity to chromosome breakage with the sensitivity peak occurring in the spermatozoa sampled 13–15 days after treatment (Period D). A neutron dose of 1.5 to 2 times greater is required to produce the same amount of radiation damage in the more mature type of cells (B) as with the D types.

Cells treated in meiotic and premeiotic stages contained fewer translocations and lower percentages of dominant lethals than postmeiotic cells. The reduction in the number of spermatozoa in the treated males in mating period G indicates that some types of spermatogonial germ cells degenerate as a result of neutron irradiation.

The relative biological efficiency of fission neutrons and 200 KV X-rays differ in mature sperm, spermatids and meiotic cells. Fission neutrons are at least six times more effective than X-ray in mature sperm; in spermatids, the difference is only two to three times larger for neutrons. In meiotic cells fission neutrons are only 1.6 times more efficient.

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AN ANALYSIS OF DATA FROM LABORATORY POPULATIONS OF DROSOPHILA MELANOGASTER

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THE detailed analysis of data from biological experiments may sometimes be thwarted by the magnitude of the computational effort involved in evaluating the variables in the statistical formulation. In those instances where a precise mathematical model can be set up, this difficulty can be overcome by the use of high-speed computers. One such application is described in this paper; it will be shown that not only can they provide a more satisfactory interpretation of numerical results, but also that a greater insight into the issues involved, and possible deficiencies in the experimental procedures may be revealed by the detail and precision of the numerical output of such gadgets.

The computer used was the "Oracle," operated by the Mathematics Panel of the Oak Ridge National Laboratory. The conversion of the maximum likelihood equations developed in the course of this work into the language of the machine (programming) was the responsibility of the first author; the various routines described here, although adequate to the task at hand, are admittedly amateurish and should be regarded only as evidence that productive effort is possible even if a programmer has limited experience and mathematical talent.

THE PROBLEM

The data analyzed are those of HEXTER (1955), which were collected to test the possible existence of single locus and/or multilocus heterosis. In brief, HEXTER found that in laboratory populations of *Drosophila melanogaster* in which certain mutant genes were present in panmixia with their wild type alleles, the frequencies of the wild and homozygous recessive phenotypes, and of the homozygous wild type and heterozygous genotypes departed from the expectations based on the HARDY-WEINBERG Law and, furthermore, that the departure from expectation was generally most pronounced in the case of the heterozygote, which appeared more often than expected. From this it was concluded that such counts might serve as evidence for heterosis.

Several questions arise at this point: (1) If the population counts deviate from the HARDY-WEINBERG Law in its simple form, do they conform with the expectations when the HARDY-WEINBERG expression is modified to include viability factors for the three genotypes? (2) Is it possible that the deviations are brought about, not by an increased viability of the heterozygote, but by a decreased via-

[1] Operated by Union Carbide Nuclear Company for the U. S. Atomic Energy Commission.

bility of the homozygous recessive class, which would be not unexpected, or by some combination of the two? (3) Can the values of these viability factors be estimated from the data available? The modification of the HARDY-WEINBERG expression required to answer these questions leads to equations that are utterly intractable by ordinary methods, hence the use of the automatic computer.

The Nature of the Data

The populations analyzed involved six different mutants: eyeless, clot, vestigial, thread, hairy, and black. Each of the six experiments was divided into two lines, one in which the initial frequency of the mutant allele was high (0.80) and another in which it was low (0.20). Counts of the two phenotypes, and genetic tests of a sample of the wild type flies to determine the relative frequencies of homozygotes and heterozygotes, were made monthly for about a year. Combining the two lines in each experiment gives a total of about 24 (22–28) counts for each experiment. Because the first few counts might deviate from the HARDY-WEINBERG distribution for extraneous reasons, which HEXTER points out, the first two in both high and low lines in each experiment were excluded from the data analyzed here.

Table 1 is a reproduction of the data in the *thread* experiment. It shows the largest deviations of all six experiments. If the first two counts of both high and low lines are disregarded, there are in the remaining 22 counts, six cases where

TABLE 1

Summary of thread experiment: Frequencies of thread and wild type flies and results of progeny tests. Frequency of recessive allele (q) *and test of goodness of fit to Hardy-Weinberg distribution* (χ^2)

	High line						Low line					
	Population count		Progeny test				Population count		Progeny test			
Count	+	*th*	+/+	+/*th*	*q*	χ^2	+	*th*	+/+	+/*th*	*q*	χ^2
1	1547	125	22	72	0.294	39.6*	1008	74	23	65	0.289	33.2*
2	1840	168	17	60	0.303	32.1*	1368	123	20	57	0.303	25.0*
3	1745	136	30	52	0.279	13.9*	2292	234	32	50	0.299	7.7†
4	2095	202	31	57	0.304	12.2*	1841	166	17	47	0.297	20.5*
5	2269	227	26	56	0.309	15.2*	2106	203	25	36	0.300	4.2‡
6	2471	198	27	58	0.281	21.3*	2020	246	37	47	0.332	1.3
7	1807	112	32	41	0.249	8.5†	1923	147	29	42	0.273	7.9†
8	2437	154	40	45	0.249	6.3‡	2794	226	36	45	0.277	5.0‡
9	2225	102	30	32	0.216	7.4†	2580	181	37	42	0.260	4.8‡
10	2601	103	47	37	0.200	4.6‡	2512	149	44	45	0.242	5.3‡
11	2329	84	47	29	0.190	1.5	2262	112	49	37	0.221	1.9
12	1952	53	57	28	0.166	0.9	2589	96	42	43	0.198	12.6*
13	2163	51	49	30	0.159	4.9‡	2773	104	48	41	0.196	7.5†

* Significant at 0.001 level.
† Significant at 0.01 level.
‡ Significant at 0.05 level.

the test of goodness of fit to the HARDY-WEINBERG distribution shows a deviation significant at the 0.001 level, four at the 0.01 level, and seven at the 0.05 level. The data from the *vestigial* experiment (not reproduced here) have out of 22 counts, six deviating at the 0.001 level, four at the 0.01 level, and three at the 0.05 level. The data from the other experiments are less extreme although each, with the possible exception of data involving the gene *clot*, presents an unexpectedly high number of counts departing from the expected ratios.

Formulation of the Maximum Likelihood Expressions

Instead of testing the distribution of the four types (two phenotypes and two wild-type genotypes) against a simple HARDY-WEINBERG distribution, the assumption will be made that relative to the frequency of the homozygous wild-type, which is arbitrarily taken as unity, the frequency of the heterozygote is modified by a factor k_1 and that of the homozygous recessive by a factor k_2. For each count, then, the gene frequency, q, of the recessive allele will be evaluated, and for all counts simultaneously, k_1 and k_2 will be evaluated for a "best fit." The six experiments are to be tested individually in this fashion in an attempt to answer the questions posed earlier.

If the relative frequencies of the three genotypes at each count are defined as $(1-q)^2$, $2q(1-q)k_1$ and q^2k_2, then the relative proportions of the four classes, the phenotypically wild type and the homozygous recessive among the total counted, and the homozygous wild type and heterozygotes among the wild types tested, become:

$$+ \quad \frac{(1-q)^2 + 2q(1-q)k_1}{(1-q)^2 + 2q(1-q)k_1 + q^2k_2}$$

$$a/a \quad \frac{q^2k_2}{(1-q)^2 + 2q(1-q)k_1 + q^2k_2}$$

$$+/+ \quad \frac{(1-q)^2}{(1-q)^2 + 2q(1-q)k_1}$$

$$+/a \quad \frac{2q(1-q)k_1}{(1-q)^2 + 2q(1-q)k_1}$$

If A, B, C, and D are the actual numbers of the above four classes, in that order, in any count, then the solution for q is obtained by equating to zero the derivative with respect to q of the logarithm of the likelihood, the latter, apart from a constant multiplier, being simply the product of the last four expressions raised to the A, B, C, and D powers, respectively. This equation is

$$\frac{\delta \log L}{\delta q} = -\frac{A+C}{1-q} + \frac{(A-C-D)(2k_1-1)}{1-q+2qk_1} + \frac{2B+D}{q}$$

$$-\frac{2(A+B)(qk_2+q-2qk_1+k_1-1)}{(1-q)^2+2q(1-q)k_1+q^2k_2} = 0$$

There is no simple algebraic solution to this equation. The computer was programmed to solve for q in a rather simple fashion by trial and error. By use of arbitrary numerical values of k_1 and k_2 at the outset and subsequently machine altered values as the program proceeded toward the final solution, the value $\frac{1}{2}$ was substituted for q, and the expression evaluated. If positive, indicating that q was too small, $(\frac{1}{2})^2$ was subtracted from the previous guess of $\frac{1}{2}$, and if too large, it was added. Successive powers of $\frac{1}{2}$ were added or subtracted until q was estimated accurately to $(\frac{1}{2})^{15}$.

Although q is estimated individually for each count, k_1 and k_2 are to be estimated for the best fit to all the counts in a given experiment. Consequently, the complete maximum likelihood expression involves the product of the individual expressions for each count. This product becomes the summation after the logarithm is taken. Where N is the total number of counts in the experiment being analyzed, the first partial derivatives of the log likelihood with respect to k_1 and k_2 are respectively,

$$\frac{\delta \log L}{\delta k_1} = \sum_1^N \left[\frac{(A_i-C_i-D_i)2q_i}{1-q_i+2k_1q_i} + \frac{D_i}{k_1} - \frac{2(A_i+B_i)q_i(1-q_i)}{(1-q_i)^2+2q_i(1-q_i)k_1+q_i^2k_2} \right]$$

$$\frac{\delta \log L}{\delta k_2} = \sum_1^N \left[-\frac{(A_i+B_i)q_i^2}{(1-q_i)^2+2q_i(1-q_i)k_1+q_i^2k_2} + \frac{B_i}{k_2} \right].$$

In principle, the steps involved in the computer program for the solution for any one set of data are the following:

1. k_1 and k_2 are arbitrarily set at some starting point within the "biologically acceptable range" (neither can be negative, nor can they be distastefully high). The gene frequency q is evaluated for each count individually from 1 to N.

2. The numerical values of the first partial derivatives with respect to the viability coefficients are obtained by summing the individual contributions from each count. A χ^2 total is computed for the entire set of data.

3. On the basis of the first partial derivatives, either k_1 or k_2, or both, is adjusted to give a better fit, and the program returns the computations to step 1.

4. When values of k_1 and k_2 have been estimated such that the most recent readjustment of both k_1 and k_2 gives a change in both of less than 2^{-20}, the system is considered to be solved and the computer calculates the χ^2 total at this endpoint. (This does not imply that the over-all accuracy of all the computations was 2^{-20}.

Although such precision could readily be achieved by the machine, we felt that the raw data did not warrant the additional time required to attain it. Consequently, the over-all accuracy of the computations was limited to give a maximum error of about 2^{-12}, or about three decimal places. Some idea of this accuracy can be obtained by noting the relative uniformity of the quotient k_1^2/k_2 within each series in Table 2).

TABLE 2

Machine output of the maximum likelihood solutions of the viability coefficients for one dummy set of data and six tables of Hexter

Experiment	Initial k_1	Initial k_2	Final k_1	Final k_2	Initial χ^2	Final χ^2	k_1^2/k_2
Dummy (D.F. = 23)	1.000	1.000	1.69791	1.23004	164.75	0.00	2.34375
	1.500	1.500	1.99277	1.69433	46.29	0.00	2.34375
	0.500	0.500	Divergent				
	1.500	0.500	0.80621	0.27737	112.13	0.00	2.34375
	0.500	1.500	Divergent				
eyeless (D.F. = 23)	1.000	1.000	1.26970	1.06655	58.64	27.49	1.51154
	1.500	1.500	1.50682	1.50210	27.52	27.49	1.51154
	0.500	0.500	0.92724	0.56880	242.57	27.49	1.51154
	1.500	0.500	0.35278	0.08233	258.67	27.49	1.51162
	0.500	1.500	Divergent				
clot (D.F. = 23)	1.000	1.000	1.14084	1.06946	35.46	26.47	1.21698
	1.500	1.500	1.29877	1.38601	38.30	26.47	1.21699
	0.500	0.500	0.88675	0.64612	210.22	26.47	1.21698
	1.500	0.500	Divergent				
	0.500	1.500	Divergent				
vestigial (D.F. = 21)	1.000	1.000	1.79321	0.70441	114.05	16.74	4.56513
	1.500	1.500	1.05899	0.24563	107.85	16.74	4.56531
	0.500	0.500	1.12628	0.27786	26.76	16.74	4.56535
	1.500	0.500	1.50952	0.49911	16.66	16.74	4.56531
	0.500	1.500	1.56164	0.53418	233.36	16.74	4.56537
thread (D.F. = 21)	1.000	1.000	1.62832	1.20081	200.82	65.51	2.20800
	1.500	1.500	1.91379	1.65878	95.86	65.51	2.20797
	0.500	0.500	1.20679	0.65958	323.26	65.51	2.20797
	1.500	0.500	0.66019	0.27920	123.30	65.51	2.20804
	0.500	1.500	Divergent				
hairy (D.F. = 19)	1.000	1.000	1.33236	1.20276	41.20	13.68	1.47589
	1.500	1.500	1.48172	1.48752	13.84	13.68	1.47591
	0.500	0.500	1.07431	0.78198	208.41	13.68	1.47590
	1.500	0.500	Divergent				
	0.500	1.500	Divergent				
black (D.F. = 17)	1.000	1.000	1.42767	1.18188	76.91	26.36	1.72460
	1.500	1.500	1.64703	1.57291	29.54	26.36	1.72461
	0.500	0.500	1.08755	0.68579	283.04	26.36	1.72462
	1.500	0.500	Divergent				
	0.500	1.500	Divergent				

Programs Used

One serious problem recognized prior to the attempt to solve these equations was that of the possible existence of multiple solutions, since it was not obvious from the form of the equations that there could exist one and only one set of values for k_1 and k_2 that would lead to a solution. This was handled empirically in several different ways. First, a "graphing" program was written that evaluated the first partial derivatives as k_1 was held constant and k_2 varied over its range, then k_1 was stepped up by a small fraction (2^{-4}) and k_2 was varied again. The process was repeated until k_1 reached its upper limit. When fed into the computer, it showed that the derivatives changed sign only once as one viability coefficient was held constant and the other varied, indicating that for any acceptable k_1 there would be one and only one acceptable k_2, and vice versa. Second, two different programs were written for maximizing the likelihood with respect to k_1 and k_2. The first was essentially a trial and error routine and the second, a modification of NEWTON's method. It was thought that if both routines arrived at the same solutions consistently, the existence of multiple solutions could be considered unlikely. Finally, both these methods were programmed to allow for a variety of starting values, of k_1 and k_2, to create the possibility of arriving at different sets of final values, if such should exist. It was discovered that there are, in fact, multiple solutions, and that these bear a simple but nevertheless unexpected relation to each other. This relation was discovered during the process of "debugging."

It is not unusual for a programmer to err in his attempt at converting the operations implied in a mathematical expression to the language understood and slavishly obeyed by the computer. Such errors are, in fact, a source of constant embarrassment and consternation to the beginner. The systematic depletion of errors from a program is known in the trade as "debugging." One effective method of debugging consists in feeding in a mock problem to which the answer has previously been determined by hand.

The data used for debugging purposes were as follows: Let $A=2700$; B, 200; C, 40; and D, 50. Simple substitution shows that there is an exact solution when k_1 is 5/4, k_2 is 2/3 and q is 1/3, the numbers for A, B, C, and D having been derived originally by substituting these fractions in the maximum likelihood expression to get an exact fit. The χ^2 should consequently be reduced to zero (or very close to it) when the best values of k_1 and k_2 are computed. However, if both k_1 and k_2 equal 1, the χ^2 value is about 6.8, and if repeated in tabular form 24 times to simulate a set of actual data, the χ^2 comes out to about 163. The first line of Table 2 shows a rather surprising result. With the initial values of k_1 and k_2 of 1.000, the χ^2 comes out correct, but the final values are not those anticipated, but are instead 1.698 and 1.230. Note, however, that the χ^2 has been reduced to zero, indicating that an exact fit has been achieved. Furthermore, different initial values (lines 2 and 4 of the table) lead to different final values but in each case the χ^2 is reduced to zero; these values also fit the maximum likelihood equation.

This occurrence of multiple solutions could have been anticipated because, as

is evident from the mechanics of computing χ^2, the minimum χ^2 for 24 identical sets of data is the same as 24 times the minimum χ^2 for a single set. There are two degrees of freedom for a single set that permit calculation of two parameters for a perfect fit, but not three. In the present example either k_1, k_2, or q can be given an arbitrary value and an exact fit obtained by adjusting the other two, as is shown by the following algebra where R_1 is the ratio of expected frequencies of wild type to homozygous recessives, and R_2 the ratio of the expected frequencies of homozygous wild type to heterozygotes:

$$R_1 = \frac{(1-q)^2 + 2q(1-q)k_1}{k_2q^2}, \quad R_2 = \frac{(1-q)}{2qk_1}$$

then,

$$1-q=R_2 2qk_1, \quad q=\frac{1}{1+2R_2k_1}, \quad \text{and} \quad R_1k_2q^2=(1-q)(1-q+2qk_1). \qquad (1)$$

Substituting,

$$R_1k_2\left[\frac{1}{1+2R_2k_1}\right]^2=\left[\frac{2R_2k_1}{1+2R_2k_1}\right]\left[\frac{2R_2k_1+2k_1}{1+2R_2k_1}\right]$$

and finally, $$\frac{R_1}{4R_2(R_2+1)}=\frac{k_1^2}{k_2}. \qquad (2)$$

If experimental ratios are substituted for R_1 and R_2, and if either q or k_1 is given any abitrary value, the value of the other of these two parameters required for a perfect fit can be obtained from expression (1), and k_2 then computed from expression (2); or if k_2 is given an arbitrary value k_1 can be computed from (2) and q from (1). For any such fit, however, the quotient k_1^2/k_2 (expression 2) depends only on the two experimental ratios corresponding to R_1 and R_2.

For any one set of data, then, consisting of four numbers A, B, C, and D, all combinations of k_1 and k_2 for which the ratio k_1^2/k_2 has the value indicated by expression (2) can provide an exact fit. The program was extended slightly by the addition of the machine computation of this ratio since it might have some relevance in the evaluation of solutions provided by the computer.

ANALYSIS OF THE DATA

Table 2 shows the output of the computer for all six tables of HEXTER. The specific routine used here was the modification of NEWTON's method for estimating two parameters attributed to FISHER by KOSHAL (1933) and GARWOOD (1941). This method was suggested to the authors by DR. ALLYN KIMBALL of the Mathematics Panel, Oak Ridge National Laboratory, to whom thanks are tend-

ered particularly because it proved to be the most efficient method of the three mentioned previously, usually leading to a final set of viability coefficients from a given starting point in about 40 seconds, whereas the other took several minutes. For each of the six experiments, five different initial values were used. In some cases, final values were not obtained because successive differences in the viability constants became larger rather than smaller (i.e., appeared to diverge). The indication of divergence does not necessarily imply that a solution is impossible from these starting values but only that arriving at the solution would probably be more time consuming than in those cases where successive values of k_1 and k_2 appeared to converge. The program included a test for such divergence such that when it occurred, a notation to that effect was recorded in the output and the computer proceeded to the next set of starting values.

When both the initial values are 1.000, the HARDY-WEINBERG expression is unadjusted, and the χ^2 computed at that initial point corresponds to the one that would be obtained by comparing the data directly with the simplest form of the HARDY-WEINBERG Law. It is seen that in every such instance where the initial values are both 1.000, the total χ^2 is reduced considerably by estimating k_1 and k_2, indicating that the modified expression, with those viability constants, is more compatible with the data than the unmodified one. Furthermore, the χ^2 values remaining after those estimates are made are not inconsistent with expectation based on the number of degrees of freedom remaining at the conclusion of the computations, except for one case, that of thread. Since the χ^2 remaining in the latter case is still rather high (65.5 for 21 D. F.), there may be other factors responsible here for the original high χ^2. In this case there may be evidence for multilocal heterosis, as HEXTER points out in his original account, with a breakdown of that system in later generations leading to an over-all heterogeneity of the data.

The final values for k_1 and k_2 are different within any set, although the χ^2 totals at the end points are much the same, indicating that these different final values fit the data equally well. Thus, despite the ample number of degrees of freedom (18–24 in the different experiments to estimate 11–14 parameters, respectively), multiple solutions were obtained here as with a single set of data. In the clot experiment, for example, equally acceptable fits are obtained if the heterozygote is assigned a viability of 130 percent and the homozygous recessive a viability of 137 percent (compared to the homozygous dominant), or if the first is assigned a viability of 87 percent and the second a viability of 64 percent. However, the values of the ratio k_1^2/k_2 (given in the last column of table 2) remain constant in any given experiment, being 1.2170 in the clot experiment. The regular agreement of those ratios within any set of iterations for a given experiment strongly suggests that the maximum likelihood solution occurs when that ratio is reached, and that it will occur at different points, depending on the values assigned to the viability factors at the start of each iteration.

The machine has thus forced to attention a circumstance completely unexpected by the authors and others with whom the problem had been discussed; namely,

the occurrence of multiple solutions. On reconsideration, however, the reason was not difficult to discover.

χ^2 for any experiment depends on the theoretical ratios R_1 and R_2 calculated for each set of data. These ratios in turn are determined by the chosen values of k_1 and k_2 and the individual values of q determined for each set. As shown by equations (1) and (2), any theoretical ratios (R_1 and R_2) whatsoever, dependent on a given k_1, k_2, and q, will also result from any other k_1 and k_2 having the same quotient k_1^2/k_2 provided that q is given a value corresponding to expression (1). Suppose, then, a minimum χ^2 results from given values of k_1 and k_2, with q being optimum for each set of data of an experiment. If we now change k_1 and k_2 so that k_1^2/k_2 remains constant, and adjust each q in accordance with expression (1), χ^2 remains the same, and is therefore still a minimum. The parameters are thus not independent, and there is in fact a fatal interdependence among them that prevents evaluation of k_1 and k_2 but permits an estimation of the ratio mentioned.

DISCUSSION

The solutions presented in Table 2 tell a number of things about the analysis of this sort of data. In the first place, it is clear that a test of goodness of fit of the actual counts against an unmodified HARDY-WEINBERG Law can tell little about the source of any heterogeneity, if such exists. In the second place, the analysis of such data without the help of an automatic computer could lead to erroneous results, since it would not be obvious that a multiplicity of solutions existed. The backbreaking labor involved in arriving at even one such solution by hand might very well preclude the possibility of the regular repetition of the analysis by different initial values. The first values derived that satisfied the maximum likelihood equations might then be taken as the proper ones. Finally, the advantage of analysis of the data with a computer is shown also by its revelation of a simple algebraic relation inherent in the maximum likelihood formulation that was not obvious (to the authors) previously.

In a sense, such automatic devices might be used in the planning of an experiment as well as in the analysis of the data from it. If, before the beginning of an experiment, it is known what sort of analysis will be used finally and if that analysis is programmed immediately, then the introduction of sample data may reveal ambiguities not obvious at the start, as it did in this particular case. Under these conditions, it might be well to modify the experiment so as to remove these ambiguities.

Of the three questions posed in the introduction, the third remains unanswered. If we had had sufficient information about the generation time, the manner of generation overlap, and had been willing to make certain additional assumptions such as, for example, that the change in gene frequency is caused entirely by these viability differences, we might have obtained from HEXTER's data the best estimates of k_1 and k_2 instead of their ratio. It seems more practical, however, to obtain

this information on relative viabilities from a simple experiment designed to test just this point.

These comments are meant as general observations and not as contradictions to the analysis of HEXTER, whose detailed consideration of the data leads to a number of interesting conclusions in other connections. We hope that this account may call the attention of other geneticists to the greater possibilities for precise, detailed, and accurate analyses of such problems by the use of computing devices, which are becoming more available.

SUMMARY

In some cases heterosis is inferred because a population appears to contain more heterozygotes than can be accounted for by ordinary genetic theory. A mathematical model based on a maximum likelihood formulation has been set up that allows an estimate to be made of the degree to which irregularities in genetic data require the assumption of heterosis. The analysis based on such a model is not practicable by ordinary methods, and for this reason an automatic computer (the Oracle at the Oak Ridge National Laboratory) was used. The output of the computer showed that the assumption that heterosis is responsible for the increased frequency of the heterozygotes is unwarranted, and that a reduced viability of homozygous classes will equally well explain such data. This result is of some interest because it shows that one of the most commonly applied tests for heterosis, a statistical test for an excess of heterozygotes in a population, is actually ambiguous even if the data should consist of a large number of replicated trials or observations.

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THE DOSAGE RESPONSE CURVE FOR RADIATION INDUCED DOMINANT LETHAL MUTATIONS IN THE HONEYBEE[1]

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HYMENOPTERA are desirable for studying dominant lethals because they provide an easy criterion for distinguishing between unfertilized and fertilized eggs as the former normally develop into haploid males while the latter become females. This characteristic has been used by STANCATI (1932), WHITING (1938), MAXWELL (1938), and HEIDENTHAL (1945) in studying dominant lethals with Habrobracon (*Bracon hebetor*).

The honeybee, another hymenopterous insect, offers the following additional advantages: a queen bee ordinarily lays fertilized eggs in worker (female) comb cells and unfertilized eggs in larger drone (male) cells; a queen normally lays over 1000 eggs per day, providing adequate numbers for statistically reliable results; sperm stored in the spermatheca of a queen may be capable of fertilization for several years. However, using the honeybee for studying dominant lethals presents some disadvantages: the large number of chromosomes ($n=16$) and their small size make this material unsuitable for cytogenetic analysis by standard techniques; each queen must be artificially inseminated if controlled matings are desired; there is normally only one reproductive female (queen) per colony.

Radiation induced dominant lethals have been attributed to those chromosome aberrations that result in bridge formation during cleavage, bridge formation usually being a sufficient condition for lethality (PONTECORVO and MULLER 1941; PONTECORVO 1941; MULLER and PONTECORVO 1942). The kind of chromosome aberration that is responsible for dominant lethals may be distinguished by the shape of the curve relating proportion of radiation induced dominant lethals to dosage (PONTECORVO 1942). While the production of dominant lethals by changes other than chromosomal (such as the disruption of DNA synthesis as suggested by VON BORSTEL 1955) or other than nuclear changes cannot be completely ruled out, it seems probable from experiments with Drosophila that most dominant lethals are due to structural changes in the chromosomes (MULLER 1940; PONTECORVO 1941; PONTECORVO and MULLER 1941; MULLER and PONTECORVO 1942; PONTECORVO 1942; DEMEREC and FANO 1944; CATCHESIDE and LEA 1945; LEA and CATCHESIDE 1945). Structural changes in the chromosomes leading to bridge formation in early cleavage could either directly or indirectly inhibit the synthesis of DNA, thus explaining VON BORSTEL's (1955) results. Any theory proposed

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to explain dominant lethals must give results consistent with the observed shape of the dosage response curve.

This paper presents data on the dosage response curve. The controls include data on the effect of fractionating the dosage and the stability of dominant lethals in spermatozoa stored in the spermathecae of queens for a year.

METHODS

Drones were irradiated with gamma radiation from a 30 Curie cobalt^{60} source (described by FIRESTONE and WILLARD 1953) at two different positions. For low dosage work three test tubes (5mm O.D.), each containing two drones placed one above the other, were strapped to a wooden positioning block ten centimeters from the source. Intensity of radiation was determined by an ionization chamber approximately the size of a drone. By correcting for the decay rate of cobalt^{60} the intensity was determined to be 57, 50, and 44 roentgens per minute respectively during the summers of 1953–55.

For high dosage work drones were placed individually in gelatin capsules taped to the positioning glass tube on a level with the center of the source. The previous method of dosimetry was not feasible at this position. A rough estimate of the intensity was obtained by assuming that there was no intensity effect and then comparing the time required to give a percent of dominant lethals equivalent to those produced at the ten centimeter position. The intensity at the position next to the source was estimated at 982, 870, and 762 roentgens per minute during the summers of 1953–55.

The shape of the dosage response curve at low doses and the fractionation effect were determined entirely from drones irradiated ten centimeters from the source, all of the radiation for the former being done in a period of four weeks in 1954. Dosage is therefore a linear function of time in these experiments, and errors in measuring the intensity would therefore not affect the conclusions. This also applies to the high dosage work which was done entirely at the position on the glass tube next to the source. This was done to determine the relative difference between the dosage required for complete dominant lethality and that required to produce inviable sperm.

Dominant lethals studied here must have been produced in mature spermatozoa because in the drone honeybee, unlike Drosophila, meiosis occurs only in the pupa stage, the sperm having migrated to the seminal vesicles and the testes being partially degenerated by the fifth day of adult life (BISHOP 1920; HACHINOHE and ONISHI 1952). Although mature spermatozoa are found in the seminal vesicles three to four days after eclosion (MACKENSEN 1955b), a satisfactory ejaculation and yield of sperm is not obtained until the eighth day or later. This delay is presumably due to necessary changes in the structure of the sexual organs described by BISHOP (1920). Only drones of sufficient age to give a satisfactory ejaculation were used in this work. When pressure is applied to the drones' abdomen, spermatozoa in the seminal vesicles are ejaculated and may be collected for artificial insemination.

Each queen for which data are reported in this paper was inseminated artificially (for techniques, see MACKENSEN and ROBERTS 1948) with pooled semen from simultaneously irradiated drones. After insemination each queen was kept in a small colony (nucleus).

Egg viability was determined by the following procedure. An empty comb was placed in the small colony containing the queen to be tested. Twenty-four hours later the comb was removed and an area containing 400 to 500 eggs was marked off with wires. Every egg in a normal worker cell in this area except those in cells bordering the wires was counted. Combs containing the counted eggs were then placed in full strength test colonies for hatching. Each test colony had its queen confined to two combs which were removed before her eggs hatched and were so manipulated that each had a normal population of adult bees and sealed brood but with no unsealed brood except that which developed from the eggs to be tested (for details of this procedure see LEE 1956). On the fourth day after counting the eggs the larvae in worker cells not bordering the wires were counted, and after pupation they were examined for the presence of drones in worker cells. Five viability tests were run for each queen (except where noted) with the test combs randomized between the various colonies. This procedure gave results with high reproducibility. In some cases counts of pupae and emerging adults were made in addition to the larvae counts.

Stock supplied by DR. O. W. MACKENSEN of Southern States Bee Culture Laboratory, USDA, was used in determining the dosage response curve. Drones of line B were irradiated and crossed to queens from line SW-1. MACKENSEN (1955a) showed that there was no common haploviable, homozygous lethal allele in these two lines. Stocks used for the other experiments were provided by DR. W. C. ROBERTS and DR. C. L. FARRAR of the North Central States Bee Culture Laboratory, USDA. Hybrid queens were crossed to drones from unrelated inbred lines. It is unlikely that any of these unrelated lines had a common lethal allele. The small variance obtained in these experiments among queens receiving similarly treated sperm further suggests that no common lethal alleles affected the results.

RESULTS AND DISCUSSION OF INDIVIDUAL EXPERIMENTS

The over-all response of the percentage of inviable eggs to dosage of gamma radiation ranging from 500 to 23,000 roentgens is shown in Figure 1. This graph is a composite of all the various experiments reported in this paper. Each circle (ten centimeter position) or triangle (positioned on the glass tube next to the source) represents the average percent of nonhatching eggs for all the different radiations at that dosage corrected for normal mortality. The number of nonhatching eggs laid in worker cells increased with increasing dosages until over 99 percent of the eggs failed to hatch at a dose of 10,900r. The curve in Figure 1 fails to reach 100 percent but approximates a plateau with over 99 percent of the eggs failing to hatch from a dose of 10,900r to a dose of 23,000r.

A few drones were found in worker cells at all dosages of 23,000r and less. In

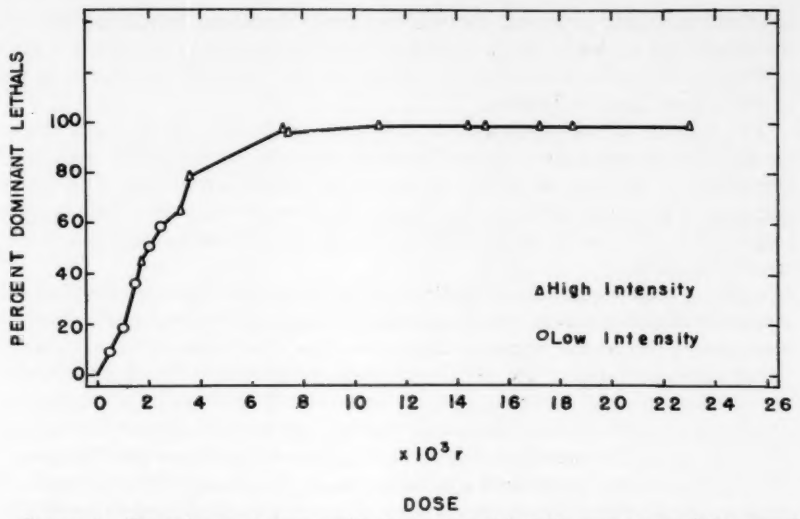

FIGURE 1.—General response curve with the percent of inviable eggs corrected for normal mortality (percent of sperm with one or more dominant lethals) plotted against the dosage of gamma radiation.

each case the number was considerably less than one percent of the number of eggs laid. It was not considered necessary to correct the data for these parthenogenic developing drones because of their very small number; however, the progeny of one queen was not included in the data because she laid a large percentage of unfertilized eggs in worker cells. Subsequent examination showed that she had very few spermatozoa in her spermatheca, presumably because of a poor insemination.

There were a few females (less than 0.1 percent of eggs laid) among progenies of queens mated to drones exposed to dosages several fold higher than the dosage producing over 99 percent inviable eggs. It is probable that these females developed parthenogenically as described by MACKENSEN (1943).

The percentage of inviable eggs for 34,000, 60,000, and 86,000 roentgens (not shown in Figure 1) was 68.0, 60.2, and 5.6 respectively. The last figure corresponds to the percentage of inviable eggs in the controls. At the above three dosages all of the progeny developed into drones (with the exception of rare females presumed to have developed parthenogenically as discussed above) showing parthenogenic development. The spermatheca of each queen was examined and all of them contained a normal number of spermatozoa. This increase in parthenogenic development at a dose of 34,000r and over is considered due to sperm inactivation.

A dominant lethal is here defined as a modification in one of the gametes which results in the death of the zygote. It was concluded that the decrease in viability of

eggs laid in worker cells over controls was due to dominant lethals because: (1) honeybee eggs normally hatch whether fertilized or not, and (2) there was a several fold difference between the dose giving nearly 100 percent nonhatching eggs and the sperm inactivation dose.

The stage of life at which most of the dominant lethals caused death was determined by counting the number of survivors (as eggs, larvae, pupae, and emerging adults) in the progenies of two queens mated to irradiated drones. This showed that nearly all the dominant lethals caused death in the egg stage, there being no appreciable reduction in the viability of larvae and pupae in the progenies from irradiated males.

Effect of dose-fractionation: The possibility of there being restitution between broken ends of chromosomes during radiation creates a possible source of error in determining the dosage response curve since time of exposure is the variable between different dosages. SAX (1939) and later workers have shown with Tradescantia microspores that the number of chromosomal rearrangements depending upon two or more breaks is decreased when the interval of time over which the dosage is applied is increased. The multihit phenomenon, half-translocation, in Drosophila oöcytes is reduced by fractionating the dosage (HERSKOWITZ and ABRAHAMSON 1956). In contrast to the above observations, experiments with mature Drosophila sperm have failed to show any change in the multihit events as a consequence of changes in the interval of time in which the dosage was administered (MULLER 1940; MAKHIJANI 1945). Apparently rejoining does not occur until after fertilization of the egg. SIDKY (1940) presented further evidence of this when he observed a translocation between sperm and egg chromosomes in Drosophila. It is thought that honeybee spermatozoa would be similar to Drosophila in this respect.

An experiment to determine the effect of dose fractionation was run with an interval of one hour between two 20 minute periods of radiation; this was compared with a continuous 40 minute period. A total dose of 2,000r was administered in each case. Each queen tested was artificially inseminated with sperm from a separately irradiated group of drones. Table 1 shows the mean of five separate viability tests on each queen. Each figure, therefore, represents data on a sample of from 2,000 to 2,500 spermatozoa.

The analysis of variance for these data is shown in Table 2. The variance is partitioned among the "within queens", "among queens within treatment", and the "fractionation effect". The within queens variance includes in addition to

TABLE 1

*Effect of fractionating the dosage of gamma radiation**

Treatment	Average percent of nonhatching eggs†				Treatment mean
Nonfractionated	55.7	58.8	58.5	52.2	56.30
Fractionated	49.6	56.2	54.0	...	53.27

* Drones were treated with a total of 2,000r given in one continuous dose or in two equal fractions separated by one hour.
† The mean of the five tests run on each queen receiving the irradiated sperm from separately irradiated drones.

TABLE 2

Analysis of variance of data from the dose-fractionation experiment

Source	d.f.	M.S.	F
Fractionation effect	1	78.70	1.54
Among queens within treatment	5	50.98	2.70*
Within queens	28	18.86	

* Indicates significance at 0.05.

sampling error the biological errors involved in testing egg viability. This latter error is relatively low compared to other errors to be discussed and was found to be consistent for all the experiments reported in this paper which ran over a period of four years. This gives confidence in the method of testing egg viability developed for these experiments. The variance among queens within treatment is the variation among the egg viability of queens inseminated with sperm from males that received equal but separate treatments of gamma radiation. This would include in addition to the previously discussed sources of error any errors in administering the gamma radiation, genetic variation in either the male or female gametes that affects egg viability, and physiological differences among queens due to either genetic or environmental effects. The fractionation effect would include all the sources of error in addition to the variation due to fractionating the dosage.

The "among queens within treatment" M.S. is used as the error in determining the fractionation effect because: (1) it is significantly larger in this experiment than the "within queens" M.S., (2) it includes any error in administering the dosage as well as the biological sources of error. In this experiment there is no significant fractionation effect. It was concluded that variations in the time of exposure in the experiment on the dosage response curve did not affect the shape of the curve.

Stability of dominant lethal mutations: The importance of the interval between radiation and testing can be ascertained by measuring the stability of dominant lethals in spermatozoa stored in a queen's spermatheca. It is possible due to the long life of a queen to extend this experiment over a longer period of time than is normally possible with nondividing animal cells. Six queens were inseminated with irradiated semen and the percentage of inviable eggs was determined soon after they began to lay. A year later they were again tested. The mean ($\bar{x}$) for the first tests and second tests conducted a year later along with the number of tests (k) conducted on each queen is shown in Table 3. We are here determining change within spermatozoa stored in the spermathecae of queens over a period of a year, so we may use as our error variance the "within queens, within years" M.S. It will be seen in the analysis of variance (Table 4 corrected for disproportion) that neither the effect of time over a period of one year (between years) nor interaction is significant. The highly significant variance among queens is ex-

TABLE 3

The stability of radiation induced dominant lethal mutations in sperm stored in the spermathecae of queens for one year

Queen	1		2		3		4		5		6	
	k	$\bar{x}$	k	$\bar{x}$	k	$\bar{x}$	k	$\bar{x}$	k	$\bar{x}$	k	$\bar{x}$
First tests	5	56.18	5	54.04	5	58.50	5	52.18	4	68.92	3	48.37
Second tests, one year later	3	57.27	3	51.00	3	60.73	3	48.73	4	66.78	4	52.25

k = number of tests conducted on each queen.
$\bar{x}$ = average percent of nonhatching eggs for each queen.

TABLE 4

Analysis of variance of data from the experiment on the stability of dominant lethal mutations

Source	d.f.	M.S.
Among queens	5	339.34*
Between years	1	1.14
Interaction (Q X Y)	5	16.99
Within queens, within years	35	20.21

* Indicates significance at 0.01.

pected since two queens received semen that had been given slightly different doses of gamma radiation.

The author is not suggesting that there was no difference between the results obtained for the two years, for spontaneous mutations could have occurred. It should be observed that the standard deviation in this experiment is 4.5 so differences of even several percent would not be detected. Unfortunately, there are not sufficient data to estimate the natural mutation rate of dominant lethals in sperm stored in the spermatheca of the queen honeybee; however, there is justification in assuming that the natural mutation rate would not be very large. It was concluded that there is no evidence for recovery from induced dominant lethals in the sperm during the one year period unless this recovery is very small and possibly compensated for by spontaneous occurring mutations. It is not necessary to take into account the time interval between radiation and testing in determining the shape of the dosage response curve.

Dosage response curve: Separate groups of drones received gamma radiation at an intensity of 50r per minute with the total dosage ranging from 500r to 2,500r at 500r intervals. Two queens were tested at each dosage, each receiving pooled semen from a separately irradiated group of drones. Normal egg mortality was estimated from two queens inseminated with nonirradiated sperm. The mean of the five separate egg viability tests for each queen is given in Table 5.

An analysis of variance was made to compare the variance among queens receiving similarly treated semen with the variance within queens. The control group was not included in this analysis. Queens receiving sperm treated with

TABLE 5

*The average percentage of nonhatching eggs for queens that received sperm irradiated with doses from 0 to 2,500r**

Dosage	0r	500r	1,000r	1,500r	2,000r	2,500r
			Percent nonhatching eggs†			
Queen A	6.80	15.32	27.66	44.64	54.61‡	68.20
Queen B	8.96	16.24	22.88	40.22	56.42	55.48
Average	7.88	15.78	25.27	42.43	55.51	61.84

* All of the radiation was done at a constant intensity of 50r per minute with dose being a linear function of time.
† The mean of the five tests run on each queen receiving treated sperm from separately irradiated drones.
‡ This queen failed to lay the required number of eggs per day. The weighted average was calculated from 3,622 eggs in numerous tests.

2,000r were excluded from this analysis because one of the queens failed to lay the required number of eggs per day. An arc-sine transformation was used to stabilize the variance and the analysis is shown in Table 6. The "among queens,

TABLE 6

Analysis of variance of data from experiment on the dosage response curve using an arc-sine transformation. Data from the queens in the control and 2,000r groups were omitted from this analysis

Source	d.f.	M.S.	F
Among treatments	3	1,569.29	34.2*
Among queens within treatments	4	45.87	4.3*
Within queens	32	10.57	

* Indicates significance at 0.01.

within treatments" M.S. will be used as the error term for the same reasons as in the experiments on the fractionation effect. The queens receiving sperm treated with 2,000r can be used in analyzing the shape of the dosage response curve since the mean for each queen is used rather than the five individual tests.

Egg viability was corrected for normal mortality on the assumption that this is independent of radiation-induced lethals.

The saturation effect caused by the possibility of inducing two or more dominant lethals in the same gamete can be corrected by assuming that dominant lethals have a Poisson distribution among the spermatozoa. This assumption is justified in testing for agreement with the linear model, where presumedly dominant lethals are the result of single breaks in the chromosomes, for there is experimental evidence that chromosome breakage has a Poisson distribution among radiated nuclei in Tradescantia (CATCHESIDE, LEA and THODAY 1946; and RICK 1940) and in the green meadow locust *Chortophaga viridifasciata* (DeG.) (CARLSON 1941); however, this correction cannot be used for testing the nonlinear model. Applying this correction the number of sperm not carrying a dominant lethal will equal e^{-m}, where m is the average number of dominant lethals per

sperm. From the average proportion of viable eggs corrected for normal mortality, values of m were calculated for each queen and appear in TABLE 7. FIGURE 2 shows the average number of dominant lethals per sperm (m) plotted against the dosage.

TABLE 7

Average number of dominant lethals per sperm

Dosage	500r	1,000r	1,500r	2,000r	2,500r
m*	0.0843	0.2414	0.5092	0.7080	1.0637
	0.0953	0.1777	0.4324	0.7486	0.7271
Av.	0.0898	0.2095	0.4708	0.7283	0.8954

* m is the average number of dominant lethals per sperm for each queen receiving irradiated sperm, and is calculated from the data in Table 5 on the assumption that normal egg mortality is independent of radiation-induced dominant lethals and that dominant lethals have a Poisson distribution.

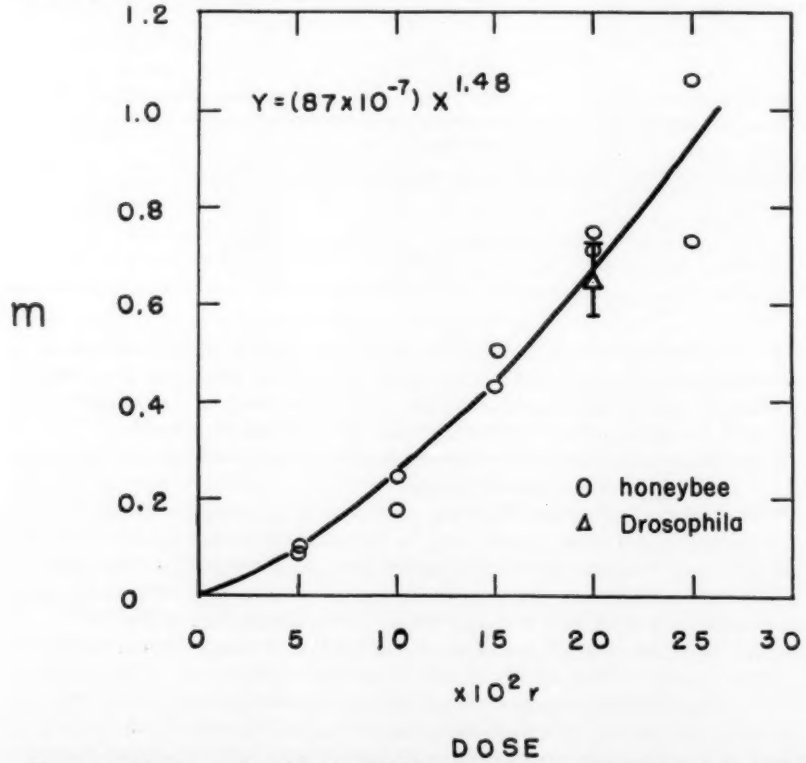

FIGURE 2.—Average number of dominant lethals per sperm (m) plotted against the dose. The curve is the least squares curve for the equation $Y = kX^n$ where X is the dose, $Y = m$, and k and n are constants. Drosophila data with 95 percent confidence interval.

If dominant lethals are linearly related to the dosage, n will equal one in the equation $m=kX^n$ where X is the dosage and k is the proportionality constant. By plotting the logarithm of X against the logarithm of m and considering the slope of the fitted regression line equal to n, n was estimated as 1.48 ± 0.38 (99 percent confidence limits), a value that differs highly significantly from 1.00. Therefore, dominant lethals are not linearly related to the dosage. However, the slope between the two lowest dosages with the log transformation approaches 1 rather than 2, indicating a linear relation as a limit. Therefore, the curve relating radiation induced dominant lethals to dosage is mixed, consisting of both a linear and a nonlinear component. The curve is in quantitative agreement with the Drosophila data of SONNENBLICK (1940), FANO and DEMEREC (1941), DEMEREC and FANO (1944), CATCHESIDE and LEA (1945), BAKER and VON HALLE (1953) and the Habrobracon data of HEIDENTHAL (1945). However, the data of RUGH (1939) indicate that the frog is considerably more sensitive than insects to radiation as measured by dominant lethals induced in sperm cells.

A direct comparison between the response of the honeybee and Drosophila to radiation as measured by induced dominant lethals was made by irradiating some Drosophila males in the same apparatus (10 cm position) in which the drones were irradiated. A total dose of 2,000r was given. Each Drosophila male was allowed to mate with an individual virgin female for 24 hours and then discarded. The average viability of each female's eggs was then determined by procedures similar to those used by CATCHESIDE and LEA (1945). There was no significant difference between the average number of dominant lethals per sperm induced in honeybee and Drosophila spermatozoa (Figure 2).

GENERAL DISCUSSION

The nonlinear component has been attributed to multibreaks in the chromosomes resulting in large deficiencies or asymmetrical interchanges (PONTECORVO and MULLER 1941; FANO and DEMEREC 1941; DEMEREC and FANO 1944; LEA and CATCHESIDE 1945), the latter probably being predominant in the honeybee due to the large number and small size of chromosomes ($n=16$). Symmetrical interchanges, a two-hit phenomenon, increase with a power greater than one but less than two with the dose (MULLER 1940; MAKHIJANI 1945) because with three or more chromosome breaks per cell, there is a disproportionately greater chance for the production of inviable recombinations. Because of this, asymmetrical interchanges leading to dominant lethals should increase at a power greater than two but approach two at low dosages. In the honeybee the shape of the dosage response curve approaches one rather than two at low dosages and has a value of only 1.48 with a dosage range from 500 to 2,500 roentgens; therefore, a linear component must be present. However, we can conclude that a very small though significant proportion of the dominant lethals is due to a multihit phenomenon at these dosages. The improper correction for the saturation effect—the correction used was based on a linear model—prevents us from calculating the exact proportion of dominant lethals due to multihits.

It would be desirable to test the relation of dominant lethals to dosage against a hypothetical, multihit model in which some of the dominant lethals are caused by two or more independently arising ionizations. Unfortunately, it is impossible to develop a meaningful multihit model because we do not know the kinetics of chromosome rejoining. The simplifying hypothesis used by CATCHESIDE (1938) and LEA and CATCHESIDE (1945) that broken ends of chromosomes rejoin at random is inadequate; however, these papers provide an excellent explanation of why symmetrical interchanges vary at approximately the 3/2 power while asymmetrical interchanges producing dominant lethals should vary at a power higher than two. While the author does not accept the quantitative exactness of the calculations of LEA and CATCHESIDE (1945), they do show that chromosome aberrations of the multihit type are sufficient to account for the nonlinear component of dominant lethals. It is not likely that many of the dominant lethals contributing to the nonlinear component of the dosage response curve are due to anything other than multibreaks in the chromosomes followed by joining to produce dicentrics or gross chromosomal deletions.

The linear component of the dosage response curve has been explained as being caused by chromosome breakage followed by sister strand fusion and bridge formation during the first cleavage division (PONTECORVO 1941; PONTECORVO 1942; MULLER and PONTECORVO 1942). PONTECORVO (1941) has shown that loss of the X or Y chromosome in Drosophila is a linear function of the dosage between 0 and 4 000 roentgens and has interpreted this loss as due to sister strand fusion followed by bridge formation. A comparison between the frequency of the losses found and the amount of change in the sex ratio caused by the same dosage suggests that a major part of the losses were lethal, presumably because of bridge formation. MULLER and PONTECORVO (1942) have shown that the abnormal sex ratio obtained when males having ring X chromosomes are irradiated is produced by death of embryos having the affected X chromosome. Apparently bridge formation is in itself a sufficient condition for lethality in about 75 percent of the cases. Considering the loss of rod X chromosomes (PONTECORVO 1941; MULLER and PONTECORVO 1942) the frequency of bridge formation due to single breaks followed by sister strand union could account for the linear component of the dominant lethal dosage response curve.

The magnitude and shape of the dominant lethal dosage response curve can be accounted for by known chromosomal aberrations in Drosophila and by analogies in the honeybee and Habrobracon; however, the question remains open whether some other mechanism, possibly acting on the mitotic process, may also account for a significant proportion of dominant lethals.

SUMMARY

The dosage of gamma radiation required to inactivate honeybee sperm is several fold higher than the nearly 100 percent dominant lethal dosage, as in Habrobracon. Nearly all the induced dominant lethals caused death in the egg stage. The proportion of dominant lethals in irradiated spermatozoa did not change after one year of storage in the spermatheca of the queen. There was no significant frac-

tionation effect, the percentage of dominant lethals being the same when 2,000r were given in a continuous dose as when given in two equal fractions separated by one hour.

The curve relating dominant lethals to dosages is in quantitative agreement with those of Drosophila and Habrobracon. It shows a highly significant departure from linearity (after correction for natural mortality and saturation), but approaches linearity at low dosages. This is consistent with the hypothesis that dominant lethals are due primarily to single chromosome breaks at low dosages and multiple break phenomena at higher dosages.

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MODIFICATION OF CHROMOSOMAL ABERRATION YIELD BY POSTIRRADIATION TREATMENT

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FOLLOWING THODAY and READ'S (1947) discovery that *Vicia faba* roots suffered more chromosomal damage when X-irradiated in the presence of oxygen than in its absence, much work has been performed to elucidate the mechanism of this "oxygen effect." The aberrations observed are usually the result of two different processes that can occur, i.e., breakage of the chromosome and the subsequent rejoining of these breaks. This is because, once formed, breaks can either remain open (one-hit aberrations) or rejoin in one of two different ways—either restituting, which is rejoining in the original configuration (no aberrations), or reuniting illegitimately with other broken ends (two-hit aberrations). It was therefore obvious that modification of either of the two processes could affect the yield of aberrations obtained with a given X-ray dose. GILES and RILEY (1950) in a series of experiments with Tradescantia microspores, the classical material for the study of radiation-induced chromosome aberrations, found that if they added oxygen very shortly after irradiation they did not get any increased aberration yield. They concluded, therefore, that the oxygen had to be present at the time of irradiation to be effective, that the increased aberration frequency obtained in oxygen resulted from a higher frequency of initial breakage, and that the post-irradiation presence or absence of oxygen had no effect on the recovery (viz., rejoining) process.

Extensive dose fractionation studies by WOLFF and coworkers (WOLFF and ATWOOD 1954; WOLFF and LUIPPOLD 1955, 1956) on X-ray-induced chromosomal aberrations in the root tips of *Vicia faba* seeds, confirmed the conclusion of GILES and RILEY that the "oxygen effect" was caused by increased breakage. However, these latter studies designed to detect any effect on rejoining led to the rejection of the assumption that there was no effect whatsoever on the recovery (rejoining) mechanism. In fact, their results indicated a postirradiation effect of oxygen on the *time* (albeit not the *amount*) of rejoining.

Since studies on the postirradiation modification of radiation-induced genetic damage are of great theoretical importance, and also since the "oxygen effect" has already been used clinically to modify the radiation sensitivity of tumors (GRAY 1956) in human beings, it seemed necessary to reinvestigate the post-irradiation effect in Tradescantia. This was thought to be particularly pertinent because some of the original data of GILES and RILEY appear to be consistent with a concept derived from the Vicia work by WOLFF and ATWOOD (1954), that there

[1] Operated by Carbide and Carbon Nuclear Company for the U. S. Atomic Energy Commission.

are two independent effects of irradiation: one on breakage and the other on rejoining.

Other conclusions from the Vicia studies tested in the present experiments on Tradescantia are that after irradiation in the presence of oxygen, the chromosome breaks produced remain open longer than when the irradiation is performed anoxically (WOLFF and ATWOOD 1954), and that the rejoining of breaks can be inhibited by the postirradiation inhibition of cellular respiration and probably of adenosinetriphosphate (ATP) production (WOLFF and LUIPPOLD 1955, 1956).

METHODS

In order to test whether or not a postirradiation effect of oxygen on aberration production existed, we decided to use the same method as previously, i.e., a dosage fractionation experiment. The rationale for this is as follows: for exchange formation to occur, two breaks must be open concurrently and be close to each other. If one considers a small volume within the nucleus and assumes that if two breaks are within this volume they will rejoin to form an exchange, then there will be a probability, p, that a break will lie within this volume and a probability, $p \times p$ or p^2, that two breaks will simultaneously lie within the volume and thus form an exchange. It is assumed that p is proportional to the number of breaks induced and thus to the dose. However, as calculated by LEA (1947), 95 percent of the breaks formed restitute. If the radiation is fractionated into two doses, so that restitution of the breaks produced by the first dose occurs before administration of the second dose, then the number of exchanges observed will be the sum of those produced by each of the doses. However, if the second dose is given before the first group of breaks restitute, then these breaks will be present concurrently with the second group and the exchanges will increase as the square of the total dose. By the inhibition of cellular respiration between two doses administered to Vicia seeds, it was possible to keep breaks from restituting. The fractionation experiments herein described were performed to test whether or not there was a similar effect in Tradescantia microspores, especially since postirradiation anoxia had been reported to have no effect in this material.

Before the experiments could be performed, however, it was necessary to determine how long a period of time was needed to ensure rejoining of the breaks produced by the first dose. SAX *et al.* (1955) reported that the breaks induced in Tradescantia microspores by a dose of 75r remained open for about one hour. In Vicia seed, this time was found to be dose dependent (WOLFF and ATWOOD 1954) and so it was necessary to perform an intensity experiment to determine how long the breaks in Tradescantia microspores would remain open with our experimental procedure. An intensity experiment is similar to a fractionation experiment in that as intensity of radiation decreases, if restitution occurs, fewer breaks will be open concurrently and the number of exchanges induced by a given dose will decrease. If the intensity is such that the total dose is administered before any appreciable number of the breaks can restitute, there will be no such decrease.

Thus the length of the plateau on an intensity curve is a measure of the time breaks remain open before restituting.

All experiments were performed on inflorescences of *Tradescantia paludosa*, (clone 3 of Sax). Four or five days after irradiation, acetocarmine smear preparations were made of microspores at the first postmeiotic mitosis. The slides were scored for two-hit chromosomal aberrations (dicentrics and rings). The X-ray source was a G.E. Maxitron operated at 250 kvp with 3 mm of Al filtration added. For treatment with vacuum or a gas, the inflorescences were placed in lucite chambers. The chambers were evacuated with a vacuum pump for 3–5 minutes before gas was introduced. In those experiments with 95 percent CO + 5 percent O_2, after evacuation, oxygen was admitted to bring the pressure up to 1/20 of an atmosphere. Then CO was added until the pressure was normal. A sodium arc lamp at a distance of 6 cm (720 ft-c as measured by a Weston Illumination meter 756 Viscor filter) was utilized to effect light reversibility of the cytochrome oxidase-CO complex. For those experiments requiring irradiation in the gas, the chamber itself was placed in the X-ray beam. All dosimetry was done with a Victoreen Radocon.

RESULTS AND DISCUSSION

The intensity curve presented in Figure 1 indicates that when the inflorescences are exposed to 200r, there is no apparent decrease in aberration yield until the irradiation was spread over 40 minutes; that is, all the breaks remained open and capable of forming an exchange for at least 20 minutes before some of them started

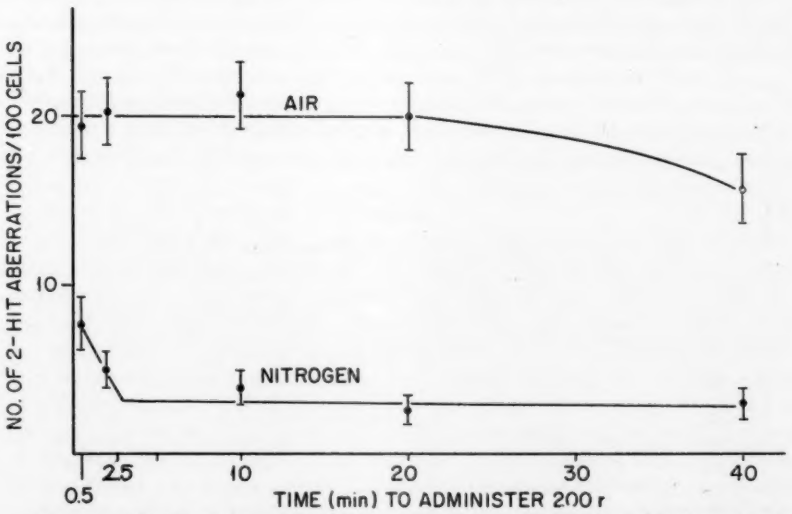

FIGURE 1.—The effect of varying the intensity on the production of two-hit aberrations induced by radiation in air and in nitrogen.

to close. When the irradiation was performed in nitrogen, a double effect was noted: (1) fewer breaks, and consequently fewer aberrations, were formed than in air; and (2) the breaks induced rejoined much faster than those induced in air. By the initial slope of the nitrogen curve, it may be concluded that breaks produced in nitrogen remained opened for a maximum of about 2.5 minutes. The general phenomenon of two independent effects of the radiation, one on breakage and one on rejoining, has already been described for Vicia (WOLFF and ATWOOD 1954) and for Trillium (DESCHNER and SPARROW 1955). The same phenomenon seems therefore to apply in Tradescantia. However, it must be noted that the time scale is different in Tradescantia since in this genus nitrogen-induced breaks rejoin in a very short time. This difference had already been apparent from the studies on postirradiation centrifugation of Tradescantia inflorescences. There it was noted for both chromosome (WOLFF and VON BORSTEL 1955) and chromatid (ANDERSON 1955) aberrations that the breaks produced in nitrogen rejoined too quickly to be moved apart by centrifugation. The same phenomenon of very rapid rejoining of breaks induced anoxically has also been observed by FETNER (1956). Although he states that the breaks remained open for long periods of time, his data from dosage fractionation studies show that even with zero time between doses, the aberration yield has approached the base line and is simply the sum of the two-hit aberrations produced by each of the half doses. This indicates that the breaks induced under conditions of partial anoxia, instead of staying open for a long time, have already rejoined before the four minutes required for the completion of the dose.

Our intensity experiments demonstrated that, unlike Vicia, the Tradescantia breaks induced in nitrogen closed too quickly to allow the easy administration of postirradiation treatments. The breaks induced in air, however, seemed to stay open for the relatively long time of at least 20 minutes. At a lower dose we expected this time to be even less (WOLFF and ATWOOD 1954) but the fractionation experiment in Table 1 shows that the breaks induced by 150r rejoin continuously over a period of about 40 minutes, after which one simply sees an aberration yield

TABLE 1

Effect of varying fractionation time on aberration yield induced by 300r

Dose I (r) in air 100r/min	Interval (min)	Dose II (r) in air 100r/min	No. 2-hit aberrations / No. cells scored	2-hit aberrations per 100 cells
0	..	...	1/200	0.5
150	0	...	49/300	16.3±2.3
300	0	...	157/300	52.3±4.2
150	5	150	148/300	49.3±4.1
150	10	150	144/300	48.0±4.0
150	20	150	119/300	39.7±3.6
150	40	150	116/300	38.7±3.6
150	80	150	102/300	34.0±3.4
150	120	150	93/300	31.0±3.2

that does not differ significantly from the sum of the aberrations produced by the two doses. For further dose fractionation experiments, therefore, a time of one hour was chosen as an adequate interval between the two doses to allow rejoining of the first breaks to occur before production of the second group. As in the Vicia experiments, immediately after the first dose, various treatments were administered to prevent rejoining until after the second dose had been given.

The results are seen in Table 2. It may be noted that in air in light the breaks induced by 150r have closed before the second dose (A). However, if after the first dose the inflorescences were placed in a vacuum in the dark, the breaks remained open (B). The interval was in the dark to prevent the formation of oxygen by photosynthesis during the course of the experiments. The experiments of WOLFF and LUIPPOLD (1955), have shown that for Vicia only very small amounts of oxygen are necessary to allow rejoining to occur, and CONGER (unpublished data) found that if Tradescantia microspores were irradiated in nitrogen in the dark, the breaks instead of closing would remain open for long times. Thus it seemed important to prevent the plants from producing their own oxygen. If between the two doses the inflorescences were subjected to a vacuum for only three minutes and then allowed to remain in nitrogen for the hour, again the breaks remained open and gave the same aberration yield as with a dose of 300r without any fractionation (C). If, however, for a control the plants were placed in air in the dark after the vacuum treatment, the breaks rejoined in the interval (D). It was also found that placing the plants in 95 percent CO and 5 percent O_2 after vacuum treatment kept the breaks open if it were done in the dark but not if done in the light (E and F). In experiment F, it should be noted that the interval between doses was increased to 75 minutes. This was because experiments performed at about the same time indicated that even when the interval was spent in air in light, incomplete rejoining occurred at the time of one hour.

These experiments are consistent with the previous Vicia experiments on postirradiation modification of aberration yield. The one thing that all the above treatments have in common is that they inhibit cellular respiration. The light reversibility of the CO effect, in particular, indicates that cytochrome oxidase activity is necessary for the breaks to rejoin.

It may be noted that each half-dose alone results in the same total aberration yield, whether or not postirradiation treatments influence the breaks to remain open longer than they would normally. This effect has also been noticed in Vicia (WOLFF 1957) and indicates that only the *time* for rejoining and not the *amount* has been affected. Therefore the fact that GILES and RILEY (1950) did not observe an increase in aberration yield when they changed the gas after a single dose of radiation does not necessarily imply the lack of an effect on rejoining. To the contrary, the experiments reported in this paper indicate that there *is* a postirradiation effect of oxygen on the rejoining of chromosome breaks in Tradescantia. It must be noted, however, that this latter effect of oxygen is to allow cellular respiration to occur and thus to allow the rejoining of breaks. The postirradiation presence of oxygen then decreases the aberration yield (if a second

TABLE 2

The effect of postirradiation treatments on the rejoining of chromosome breaks in Tradescantia

Dose I (r) in air 200r/min	Treatment for 1-hr interval	Dose II (r) in air 200r/min	Number 2-hit aberrations: Number cells scored Expt. 1	Number 2-hit aberrations: Number cells scored Expt. 2	2-hit aberrations per 100 cells	Expected yield if Dose I and Dose II are additive
	A					
0	Air in light	0	0/150	.....	0.0	...
150	Air in light	0	17/116	51/350	14.6±1.8	...
150	Air in light	150	81/237	103/300	34.2±2.5	29.2
	B					
150	Vacuum in dark	0	13/100	50/300	15.7±2.0	...
0	Vacuum in dark	150	22/150	43/300	14.5±1.8	...
150	Vacuum in dark	150	75/150	124/250	49.7±3.5*	30.2
0	Vacuum in dark	0	1/200	.....	0.5	...
300	Vacuum in dark	0	.....	106/200	53.0±5.1*	...
	C					
150	Vacuum 3 min then N_2 in dark	0	63/300	.....	21.0±2.6	...
0	Vacuum 3 min then N_2 in dark	150	34/300	.....	11.3±1.9	...
150	Vacuum 3 min then N_2 in dark	150	54/300	.....	51.3±2.4*	32.3
0	Vacuum 3 min then N_2 in dark	0	0/150	.....	0.0	...
	D					
0	Vacuum 3 min then air in dark	0	0/150	.....	0.0	...
150	Vacuum 3 min then air in dark	0	52/300	.....	17.6±2.4	...
0	Vacuum 3 min then air in dark	150	35/200	.....	17.5±3.0	...
150	Vacuum 3 min then air in dark	150	123/300	.....	41.0±3.7	36.1
	E					
150	95% CO + 5% O_2 in dark	0	38/200	.....	19.0±3.1	...
0	95% CO + 5% O_2 in dark	150	52/300	.....	17.3±2.4	...
150	95% CO + 5% O_2 in dark	150	155/300	.....	50.7±4.2*	36.3
150	95% CO + 5% O_2 in light	0	46/300	.....	15.3±2.3	...
0	95% CO + 5% O_2 in light	150	48/250	.....	19.2±2.8	...
150	95% CO + 5% O_2 in light	150	132/350	.....	37.4±3.3	34.5
	F†					
150	Air in light	0	42/300	.....	14.0±2.2	...
300	Air in light	0	135/244	.....	55.2±4.8*	...
150	Air in light	150	105/300	.....	35.0±3.4	28.0
150	95% CO + 5% O_2 in light	150	106/300	.....	35.3±3.4	28.0
150	95% CO + 5% O_2 in dark	150	141/300	.....	47.0±4.0*	28.0

* If the breaks induced by Dose I remain open to rejoin with those of Dose II, the aberration yield should be the same as that observed after an unfractionated dose of 300r.

† Interval 75 minutes.

dose is given) which is the opposite result from that ordinarily called "oxygen effect." We therefore believe that it is erroneous to interpret our data on the post-irradiation effect of oxygen as indicating that the "oxygen effect" of increased damage results from an effect on rejoining (BAKER 1955). We also believe that the "oxygen effect" of increased aberration yield has been correctly stated by GILES and RILEY to be an effect on the breakage of the chromosomes.

These results have been confirmed both in Drosophila and in Allium. ABRAHAMSON (1958) working with Drosophila oocytes found that if oxygen were present between two doses, the breaks from the first would close before the second dose was given; whereas if the flies were kept in nitrogen for the interval, then the breaks remained open (i.e., capable of forming an exchange). COHN, working with onion seeds (1956) found that CO in the dark would keep breaks open but that in the light it would not.

In Drosophila, however, the *amount* of rejoining as well as the *time* seemed to be affected. This was indicated by the observation that the aberration yield induced by a single dose was increased if the breaks were kept open by postirradiation treatments of nitrogen to females (ABRAHAMSON 1958) or cyanide to males (SOBELS 1957).

In Vicia where it is feasible to treat with solutions of inhibitors, in addition to gases, similar experiments (WOLFF and LUIPPOLD 1955) with dinitrophenol as an uncoupler of oxidative phosphorylation from respiration, have indicated that ATP is necessary for the rejoining of breaks. It has also been shown (WOLFF and LUIPPOLD 1956) that the addition of exogenous ATP stimulates rejoining.

The experimental work with Vicia led to the postulation (WOLFF and ATWOOD 1954; WOLFF and LUIPPOLD 1956) that radiation independently affects the number of chromosome breaks in the cell and the time that they will remain open. Oxygen present at the time of irradiation increases the amount of damage. This is reflected both in an increased number of breaks and in their remaining open longer. In the presence of ATP produced by respiration, however, recovery can occur and the breaks can rejoin. If the respiration is inhibited after irradiation (this can be done by anoxia), then little ATP is produced and the breaks cannot rejoin. This has been represented schematically in Figure 2. The presence of a "rejoining system" has been postulated to account for both the independence of the time of rejoining from the number of breaks induced and the dependence of this time on radiation dose.

The predictions of this theory, in addition to fitting the results observed in Vicia, Tradescantia, Allium, and Drosophila, also fit such diverse phenomena as the reverse "oxygen effect" of BEATTY *et al.* (1956) and the results obtained by GILES and RILEY (1950) when they changed gases during irradiation. In the former case it may be argued that after long periods in helium a state of complete anoxia results and breaks induced in Tradescantia remained open to rejoin with breaks induced at a later time. This then raises the aberration yield under anoxic conditions and gives a reverse oxygen effect (see SWANSON and KIHLMAN 1956). In the latter case GILES and RILEY observed that when they administered the

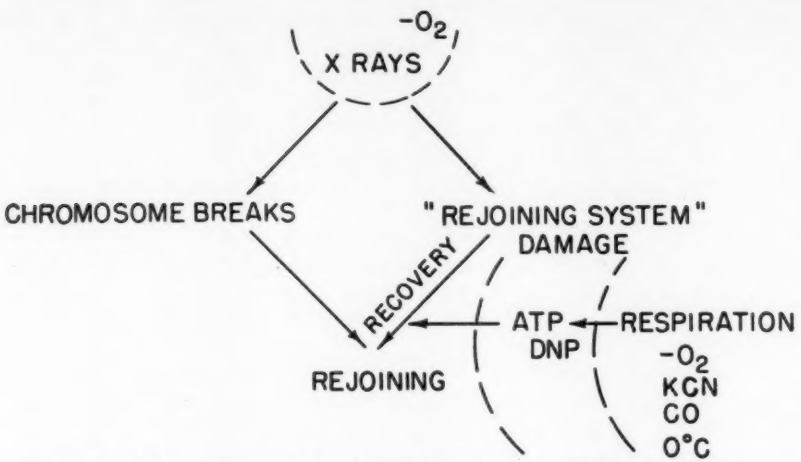

FIGURE 2.—Schematic representation of effects of radiation on production of X-ray induced chromosome exchanges.

first half of the dose *in vacuo* and the second half in oxygen, they obtained a lower aberration yield than when the gases were administered vice versa. This is similar to the effect noted by WOLFF and ATWOOD (1954) in Vicia, when the first dose was administered anoxically. It has been argued that when the first irradiation is *in vacuo*, because of less damage to the "rejoining system," the breaks induced by the first half of the dose rejoin before the second half has been finished. When the first part of the dose is administered in oxygen, the breaks induced do not rejoin until after all the breaks are present concurrently in the system. Thus the aberration yield is increased from what is observed in the opposite case. This argument can be used as an alternative explanation of the effect observed in Tradescantia by GILES and RILEY which they attribute to the fact that they could change from vacuum to oxygen much faster than they could change from oxygen to vacuum.

SUMMARY

In Tradescantia microspores, postirradiation treatments can modify the numbers of chromosome exchanges induced by X-rays. The postirradiation inhibition of respiration by anoxia or carbon monoxide in the dark prevents the rejoining of breaks and consequently allows breaks to remain open and capable of forming exchanges with breaks induced later. The results with Tradescantia are qualitatively similar to the results obtained with Vicia in that irradiation in the presence of oxygen produces more breaks than does anoxic irradiation and that for rejoining cellular respiration (the production of ATP) is required.

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CYTOGENETICS OF INTERSPECIFIC HYBRIDS, LYCOPERSICON ESCULENTUM MILL. × L. HIRSUTUM HUMB. AND BONPL.[1]

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THE genus Lycopersicon has been divided by MULLER (1940) into two subgenera:

a. *Eulycopersicon*, to which *L. esculentum* Mill. (cultivated tomato) and *L. pimpinellifolium* Mill. (currant tomato) belong. These species are red-fruited and lack pseudostipules.

b. *Eriopersicon*, to which *L. peruvianum* Mill., *L. cheesmanii* Riley, *L. glandulosum* C. H. Mull. and *L. hirsutum* Humb. & Bonpl. belong. These species are green-fruited and have pseudostipules.

LUCKWILL's (1943) classification is very similar to that of MULLER except that LUCKWILL recognizes an additional species, *L. pissisi*, which in MULLER's treatment is *L. peruvianum* var. *humifusum*.

The wild species, especially *L. hirsutum*, were intensively studied for disease resistance by ALEXANDER (1942); PORTE, DOOLITTLE and WELLMAN (1939); KIKUTA and FRAZIER (1947); and for flesh color by LESLEY and LESLEY (1947). MACARTHUR and CHIASSON (1947) studied the crossing relations of *L. esculentum* with the rest of the species and made preliminary observations of meiosis in pollen-mother cells of the F_1 hybrids.

In none of these, nor any other works, however, were segregating generations subjected to detailed genetical study, which is so important to breeding work. To understand the segregating generations it is necessary to study cytologically the chromosome behavior in the hybrid. These studies may furthermore contribute to our understanding of the cytotaxonomy and evolution of these species.

With these objectives in mind the present project was undertaken to investigate the relationships between *L. esculentum* and two botanical forms of *L. hirsutum*.

MATERIALS AND METHODS

The following stocks for these studies were provided by DR. CHARLES M. RICK.

Stocks of L. esculentum

Line a. This is a line derived from the commercial variety San Marzano, with the marker genes Woolly (*Wo*), dwarf (*d*) and male-sterile$_{10}$ (ms_{10}), known to be linked on chromosome 2 (RICK and BUTLER 1956).

[1] Part of a thesis submitted to the Graduate Division of the University of California in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Genetics.

Line b. This was derived from San Marzano also, and has the marker genes anthocyaninless (*a*), potato leaf (*c*), lutescent (*l*), and male-sterile$_{10}$ (ms_{10}).

Line c. This is the commercial variety Pearson, with the self-pruning (*sp*) and male-sterile$_2$ (ms_2) markers.

These three stocks were used as female parents because (1) the male-sterility eliminated the need for emasculation, and (2) the crosses cannot be made with *L. hirsutum* as the pistillate parent.

Stocks of L. hirsutum

Two forms of this species were used—the typical form, and *L. hirsutum f. glabratum*. The latter was represented by RICK's collection from Rio Pastaza at Bãnos, Ecuador, and the former by his collection from Rio Chillon, Peru. For the sake of simplicity these will be referred to hereafter by their respective locality names.

Chillon: This collection is of the typical form of *L. hirsutum*. It is very hairy, and has a thick, brittle, green stem. Chillon is sensitive to daylength and flowers only under 12-hour or shorter daylengths. When days are longer the flower buds dry and drop off at various stages. In the later part of May, June, July, and early August, it does not flower. It is self incompatible.

Baños: The collection from Baños is less hairy, and its stem is shorter and more elastic than that of Chillon. Young leaves and stems have a dark purplish color on the side exposed to sunlight while the shaded side is green. The inflorescence is not normally subject to this color variation. The leaves have a darker green color than those of Chillon. The leaflets vary in length, but are definitely shorter than those of Chillon. Baños is nonsensitive to daylength. It is self compatible and sets fruits abundantly both in the greenhouse and in the field at Davis, California. It is less vigorous than Chillon.

Hybridization of the species

The usual procedures were followed in making and tagging the crosses. One tenth of one percent Indolebutyric acid was used on pedicles and calyxes to induce fruit set, since all untreated crosses failed. In August and September 1951, 600 flowers were pollinated in each of the three *esculentum* lines—300 with Baños pollen and 300 with Chillon pollen.

Selfing and backcrossing

Flowers of F_1 *L. esculentum* × Baños were selfed in an insect-proof screen house. As controls eight pure *esculentum* plants heterozygous for *Wo* and *d* also were artificially selfed in the same screen house. Backcrosses were made on *esculentum* male-sterile plants in an isolation plot. If the male-sterile plants were in the main plot (in company with fertile plants), they were covered with 3′ x 3′ x 3′ cages covered with fine cheesecloth to isolate them from contaminating pollen.

F_1's of *L. esculentum* × Chillon were used for backcrosses on *esculentum* male-sterile plants both in the field and the greenhouse. These F_1 plants could not be selfed because they are self incompatible.

Chromosome morphology

Young buds of *L. esculentum*, Baños, Chillon, F_1 *L. esculentum* × Baños and F_1 *L. esculentum* × Chillon were fixed in 3:1 alcohol-acetic acid. After fixation at room temperature for twenty-four hours the buds were washed in several changes of distilled water, mordanted (method by MARTA WALTERS, quoted by BROWN 1949) for half an hour in four percent iron-alum solution, washed thoroughly in several changes of distilled water and stored at 32°F where they remained in good condition for more than a year.

Permanent slides were prepared according to the method of BROOKS, BRADLEY and ANDERSON (1950).

Chiasma frequency

Another group, comprising five or six plants each of *L. esculentum*, Baños, Chillon, F_1 *L. esculentum* × Baños and F_1 *L. esculentum* × Chillon, was raised under the uniform conditions of a greenhouse. Buds from three plants of each kind were fixed in the middle of March. Fifty or more cells were observed from each plant for determination of the chiasma frequency for the whole complement, and 40 or more were observed for chiasma frequency of the second chromosome.

Pollen study

Freshly opened flowers or those in the act of opening were collected between 9 A.M. and 10 A.M. from field grown plants of the parental species and their F_1's. Particular care was taken to collect the material when the temperatures of the week before collection were not high enough to induce pollen abortion. Pollen was examined immediately after collection.

RESULTS

Fruit set

As mentioned earlier, no fruits set in these species crosses without the application of Indolebutyric acid. Even after such treatment fruit set was quite low, 6.3 to 14 percent in *L. esculentum* × Baños and 2.7 to 5.3 percent in *L. esculentum* × Chillon.

No control crosses were made in the field for comparisons; yet, it has been the experience that under comparable conditions at Davis *L. esculentum* has a fruit set above 70 percent. The *hirsutum* pollen—either of Baños or Chillon—was more successful in fruit set on Line c. A statistical analysis by the heterogeneity test showed that this differential receptivity to Baños pollen of Line c in contrast to Line a or b is highly significant. There was no significant difference when Chillon pollen was used; but the trend was in the same direction.

Furthermore, the difference in receptivity of the same lines of *L. esculentum* to pollen of the two *L. hirsutum* forms was highly significant. Under greenhouse conditions of 70°F during the day and 60°F at night, however, the percentage of fruit set was increased to 76.6 percent and there was no significant difference between interspecific and intraspecific crosses.

Seeds per fruit

The majority of fruits from interspecific crosses were found to contain seeds with aborted embryos. Only a few fruits had fully developed seeds, and these were present in small numbers. *L. esculentum* × Baños had 4 to 20 seeds per fruit while *L. eculentum* × Chillon had 4 to 11 seeds per fruit.

When field crosses were made in the summer of 1951, pure *L. esculentum* or *L. hirsutum* was not selfed for comparison. But in the following summer Baños was selfed, producing 50.4 ± 2.5 seeds per fruit. In Pearson (Line c) the average number of seeds per fruit varies from 100 to 250, while in San Marzano (Lines a and b) the average number of seeds per fruit is about 100. The trend of seeds per fruit is in the same direction as that of percentage fruit set in these interspecific crosses. Here again, the number of seeds per fruit in Line c × Baños or Line c × Chillon is higher than in the rest of the crosses; furthermore, when Baños is used as the pollen parent the seed set tends to be higher.

Under greenhouse conditions the interspecific crosses still have significantly lower seed set than either parent, though the yield is higher than that under field conditions.

Segregation of genes

Anthocyaninless (a) *and Potato-Leaf* (c): The segregation of these two characters was scored in the greenhouse during July and August. In order to avoid crowding seedlings were classified and removed as soon as they expressed these characters clearly. The backcross results are summarized in Table 1(a).

It is obvious that the χ^2 values calculated according to 1:1:1:1 expectation give a good fit with the expectation on the hypothesis of independent assortment of the two characters. However, when the families are combined the deviations from the expected 1:1:1:1 ratio are on the borderline of significance, the probability being .04. Genes *a* and *c* are definitely known to be located on different chromosomes (BUTLER 1952). The pollen abortion in the *L. esculentum* × Baños F_1's, as noted in the cytological section, is not higher than that of either parent. Furthermore, germination in the backcross seeds is not lower than that of *L. esculentum* or Baños. A possible explanation for the above deviation, therefore, is selective elimination of the recombination types (+*c*) in an early zygotic stage.

The segregation of individual genes in all four F_2 families confirms the 3:1 expectation. At the same time the assortment of the two genes is independent, indicating no systematic disturbance [Table 1(b)].

Along with the above segregating progenies, the backcross *L. esculentum* × (*L. esculentum* × Chillon) was grown for the same gene segregation. This gave

TABLE 1

Segregation and assortment of a *and* c *in the hybrids of* L. esculentum × L. hirsutum

Family no.	++	*a* +	+ *c*	*ac*	Total	χ^2	P
a. Backcross *L. esculentum* × (*L. esculentum* × Baños)							
BP_2	26 (31.25)	29 (31.25)	30 (31.25)	40 (31.25)	125	3.54	.8–.3
BP_3	79 (73.)	69 (73.)	61 (73.)	83 (73.)	292	4.05	.3–.2
BP_4	22 (22.75)	22 (22.75)	19 (22.75)	28 (22.75)	91	1.88	.7–.5
BP_5	44 (35.50)	37 (35.50)	26 (35.50)	35 (35.50)	142	4.65	.2–.15
Total	171 (162.50)	157 (162.50)	136 (162.50)	186 (162.50)	650	8.35	.04
b. F_2 *L. esculentum* × Baños							
P_1	124 (141.75)	53 (47.25)	59 (47.25)	16 (15.75)	252	5.85	.15–.1
P_2	76 (76.50)	19 (25.50)	32 (25.50)	9 (8.50)	136	3.34	.3–.2
P_4	54 (47.81)	13 (15.94)	12 (15.94)	6 (5.31)	85	2.41	.5–.3
P_9	50 (51.75)	25 (17.25)	11 (17.25)	6 (5.75)	92	5.82	.2–.1
Total	304 (317.81)	110 (105.94)	114 (105.94)	37 (35.31)	565	1.45	.7–.5
c. Backcross *L. esculentum* × (*L. esculentum* × Chillon)							
BC_1	165 (153.)	158 (153.)	117 (153.)	172 (153.)	612	11.93	<.01

Figures in parentheses are Mendelian expectations.

an opportunity to detect discrepancies, if any, between the two pollen parents—Baños and Chillon. In this progeny the assortment of *a* and *c* is not normal, since the probability of a fit as poor as that observed is less than .01. Interestingly enough, class *a c* is excess, while class + *c* is deficient, just as in the backcross *L. esculentum* × (*L. esculentum* × Baños). The deviations in the present case, however, are more extreme than those of the Baños backcrosses.

Self-pruning Habit (sp) *and Male-Sterile*$_2$ (ms_2): Of 600 plants of the *L. esculentum* × (*L. esculentum* × Chillon) backcross population in the field, 591 survived while in the *L. esculentum* × (*L. esculentum* × Baños) backcross, 177 plants out of 405 withered and died (SAWANT 1956). The same behavior was shown by the F_2 of *L. esculentum* × Baños, with 66 plants being lost in a total of 210. The results are summarized in Table 2a.

TABLE 2a

Assortment of sp *and* ms_2 *in* L. esculentum × L. hirsutum *crosses*

Family no.	++	*sp* ms_2	*sp* +	+ ms_2	Total	χ^2	P
BC_{10}	140	142	182	127	591	11.55	< .01
	(147.75)	(147.75)	(147.75)	(147.75)			
BP_{20}	45	47	97	40	229	37.25	<.001
	(57.25)	(57.25)	(57.25)	(57.25)			
P_{19}	67	9	44	24	144	13.46	<.001
	(81)	(9)	(27)	(27)			

In *L. esculentum* × (*L. esculentum* × Chillon) backcross the recombination class *sp* + is in great excess, whereas the other recombination class, + ms_2 is deficient. It is interesting to note that the parental combinations, + + and *sp* ms_2, are less than expected, though they do not show significant deviations. Nevertheless, these observations contradict the now classical hypothesis that recombination classes in species crosses tend to be deficient and parental classes excessive.

These results can be explained to some extent in terms of abnormal segregation of these individual genes (Table 2b). In the backcross *L. esculentum* × (*L. esculentum* × Baños) we find that the probability values are lower than those in Chillon backcross, and the deviations are in the same direction as in the Chillon backcross (Table 2b).

Woolly (Wo) *and Dwarf* (d): The results for these characters are tabulated in Table 3. P_{37} is an exceptional F_2 family of the *L. esculentum* × Baños cross, which produced 70 homozygous woolly plants with a good fit to 3**Wo**:1 + ratio, the *WoWo* plants evidently being viable.

It is obvious that in these families assortment of the two characters is not independent, since the probability values are less than .001. When the χ^2's, are apportioned to the appropriate contributing factors, linkage proves to be the main source of the deviations. Families BWod–7 and Wod × acldry, being

TABLE 2b

Segregation of sp *and* ms_2 *in* L. esculentum × L. hirsutum *crosses*

Family no.	+	ms_2	P χ^2	P	+	*sp*	χ^2	P
BC_{10}*	322	269	4.75	.05–.02	268	323	5.12	.05–.02
	(295.50)	(295.50)			(295.50)	(295.50)		
BP_{20}†	142	87	13.2	<.001	85	144	15.20	<.001
	(114.50)	(114.50)			(114.50)	(114.50)		
P_{19}‡	106	38	.15	.7–.5	91	53	10.7	<.001
	(108)	(36)			(108)	(36)		

* BC_{10}—backcross *L. esculentum* × (*L. esculentum* × Chillon).
† BP_{20}—backcrosses *L. esculentum* × (*L. esculentum* × Baños).
‡ P_{19}—F_2 *L. esculentum* × Baños.

TABLE 3

Assortment of Wo *and* d *in* F_2 *and backcross of* L. esculentum × L. hirsutum *and pure* L. esculentum

Family no.*	++	*Wo d*	*Wo* +	+ *d*	Total	χ^2	P
B Wod-7	72	65	29	34	200	28.12	.001
	(50)	(50)	(50)	(50)			
Wod × acldry	224	212	114	96	646	80.51	.001
	(161.5)	(161.5)	(161.5)	(161.5)			
BP_{37}	668	686	286	322	1962	285.30	.001
	(490.5)	(490.5)	(490.5)	(490.5)			
P_{37}	147	127	308	15	597	28.65	.001
	(111.94)	(111.94)	(335.81)	(37.31)			
BC_{25}	336	328	142	94	900	208.80	.001
	(225)	(225)	(225)	(225)			
BC_{27}	335	330	140	148	973	163.40	.001
	(243.25)	(243.25)	(243.25)	(243.25)			

* B Wod-7 is backcross of $\frac{Wo\ d}{++}$ to double recessive $\frac{+\ d}{+\ d}$ female parent. It is pure *esculentum* family.
Wod × acldry is reciprocal of the above cross.
BP_{37} is backcross of F_1 *L. esculentum* × Baños to double recessive *L. esculentum* female parent.
P_{37} is F_2 of *L. esculentum* × Baños cross.
BC_{25} and BC_{27} are backcrosses of F_1 *L. esculentum* × Chillon to double recessive *L. esculentum* female parent.

homogeneous, are combined in one group; BC_{25} and BC_{27} form another group. From these, the crossing over values are calculated by means of the maximum likelihood method.

Table 4 shows that crossing over in *L. esculentum* × Baños hybrids is nearly the same when calculated from the backcross and F_2 data. Comparisons of linkage values in pure *L. esculentum*, Baños hybrids and Chillon hybrids made by the homogeneity test show that the comparison between *L. esculentum* × Chillon hybrid and *L. esculentum* is significant (P=.05); the other comparisons are of the same order.

TABLE 4

Summary of cytological observations of L. esculentum, L. hirsutum *and their hybrids*

	Crossover values		Average no. of chiasmata		Percent pollen abortion
	Backcross	F_2	Whole complement	Second chromosome	
L. *esculentum*	32.27±1.61	.....	17.91	1.77	7.09
Baños	.....	.....	17.14	...	6.79
Chillon	.....	.....	17.02	...	8.32
F_1 *L. esculentum* × Baños	30.98±1.04	31.00±2.03	17.19	1.74	7.41
F_1 *L. esculentum* × Chillon	27.98±1.04	.....	14.35	1.57	14.79
L.S.D.	.....	.....	.46*	.10*	1.67*
	.....	.....	.61†	.13†	2.21†

* At five percent level.
† At one percent level.

Cytology

The morphology of *L. esculentum* pachytene chromosomes has been described in detail by BARTON (1950) and BROWN (1949). The observations of these workers and those of the present study are in agreement. Mention should be made at this point that the tomato chromosomes are differentiated not only into chromatic and achromatic zones (BROWN 1949) but also that each zone is further differentiated into minute regions having differential stainability.

The meiotic divisions in the pollen mother cells of Baños and Chillon are essentially the same. No unusual structural configurations—translocations, inversions, multivalents, univalents or non-homologous pairings—are evident. The nucleolus in Chillon looks slightly smaller than that of *L. esculentum*, and the chromosomes of Chillon seem to stain lightly, indicating that they might differ from those of *L. esculentum* with respect to differentiation into chromatic and achromatic zones.

Cytology of F_1's: No attempt was made to study leptotene and zygotene. In pachytene pairing was observed to be complete. No rings, cross-shaped configurations, nor loops were observed.

It is interesting to note that in the homologues of a bivalent in these F_1's there is no difference in the stainability, such as is evident when the two parents are observed separately. Other workers have found evidence that the chromaticity of chromosomes is a gene controlled character but subject to the influence of the environment. Under ordinary temperature conditions the chromosomes of Trillium do not show any differentiation, but at 0°C and 5°C they clearly differentiate along their lengths into chromatic and heterochromatic regions (DARLINGTON and LA COUR 1940). Since there is cyclical correlation between gene reproduction and nucleic acid attachment, these authors conclude that staining ability—nucleic acid starvation or accumulation—is a gene controlled character. The difference in stainability in our case, then, presumably refers to physiological differentiation of the chromosome and not to the gene order. Its alteration in the F_1 shows that it does not necessarily represent a permanent structural difference.

Although the meiotic divisions are normal in gross aspects, when chiasma number for the whole complement as well as for the second chromosome are studied, certain significant differences are found, as noted in Table 4. The differences between mean chiasma number per full complement, of Baños, Chillon and F_1 *L. esculentum* × Baños are not significant, whereas the rest of the comparisons show significant differences. It is of particular interest to note that the mean chiasma number of F_1 *L. esculentum* × Baños is significantly lower than that of *L. esculentum* but not lower than that of Baños. The chiasma number of F_1 *L. esculentum* × Chillon, on the other hand, is very significantly lower than that of either *esculentum* or the Chillon parents. Comparing F_1 *L. esculentum* × Chillon with F_1 *L. esculentum* × Baños it is noticed that the former has a very significantly lower chiasma number.

The observations on the number of chiasma in the second chromosome sub-

stantiate, in part, the above results. In this case only *L. esculentum*, F_1 *L. esculentum* × Baños and F_1 *L. esculentum* × Chillon were studied. There is no significant reduction in chiasma number in F_1 *L. esculentum* × Baños as compared to pure *L. esculentum* but F_1 *L. esculentum* × Chillon has a significantly lower chiasma number than *L. esculentum* or F_1 *L. esculentum* × Baños.

It is clear from the comparisons of the differences in pollen abortion with the L.S.D. (Table 4) that the only significant difference is between F_1 *L. esculentum* × Chillon and the rest.

DISCUSSION

Nature of reproductive isolation between L. esculentum and L. hirsutum

Pollinations made under field conditions gave a very low set of fruit and seed in interspecific crosses. The reproductive isolating mechanism seems to be governed by several genes which interact disharmoniously, resulting in the abortion of a large portion of the embryos. Furthermore, there is an interaction between genotypes and the environment of the parental species, since under greenhouse conditions the seed set is improved and the fruit set is as good as that in the intraspecific crosses. The difference between field crosses and greenhouse crosses makes it difficult to infer the exact nature of the isolation mechanism operating under natural conditions. Neither field nor greenhouse conditions duplicate exactly the native habitat of these species in Ecuador and Peru. In the study of wheat hybrids Love (1941) has shown that unless definite criteria are established by intraspecific crossing relationships, it is difficult to decide on the relative significance of any individual criterion in determining the species relationships. The present study has revealed, moreover, that success in interspecific crosses can be influenced not only by the environment, but also by the genotype of the parents, for within *L. esculentum*, San Marzano and Pearson exhibit different crossability with Baños or Chillon. As a result, crossability under artificial conditions has to be considered with caution in deciding species relations.

The isolation barrier between *L. esculentum* and Baños appears to be comparatively weaker than that between *L. esculentum* and Chillon. This is true not only for crossing relations but also for chiasma frequency, pollen abortion, linkage value, seed fertility of F_1's, and segregation in F_2 and backcrosses. In the present case the two processes of reproductive isolation and morphophysiological differentiation go hand in hand. The relationships revealed by cytogenetic methods are strengthened by physiological and morphological comparisons. Thus, Chillon is sensitive to daylength and it is self incompatible, while Baños, like *L. esculentum*, is nonsensitive to daylength and is self compatible. Morphologically Baños is like *L. esculentum* by being less hirsute than Chillon, and it resembles *L. esculentum* more than Chillon does in its unfolding of calyx, the size of the plant and the exertion of stigma. In conclusion, all the criteria that were applied consistently indicated a closer relationship between *L. esculentum* and Baños than between *L. esculentum* and Chillon.

Reproductive isolation between L. esculentum *and Baños*

The nature of reproductive isolation between these two entities is of special significance. Isolation barriers in most cases seem to be controlled by a series of genes. Thus, STEBBINS (1950) states: "The nature of interspecific isolation barriers precludes the possibility that species usually arise full-fledged at a single step barring the catacylsmic event of polyploidy." The withering in the *L. esculentum* x Baños hybrids and in their segregating generations, however, is controlled by a pair of complementary genes which have no effect in the pure species (SAWANT 1956). All the F_1's and nearly half of the F_2 and backcross plants wither and die under field conditions. Such an isolation barrier can readily arise by two mutations to develop a pair of complementary factors. If the two entities involved have already diverged in their taxonomic characters, the incorporation of these complementary factors would provide an effective barrier to gene exchange between the entities, thereby providing further differentiation and full speciation.

The second difference between the *esculentum* hybrids of Chillon and Baños lies in the stage at which these isolation barriers operate. In the hybrids with Baños the latter operate in the sporophyte, whereas in Chillon hybrids they operate mainly in the gametophyte and to some extent in an early sporophytic stage (SAWANT 1956).

Segregating generations

The results of this investigation show that in backcrosses and F_2 progenies all six marker genes except *sp* and ms_2 segregate normally when considered individually, the deviations being small and spurious. When combined segregation of these genes is studied, however, the deviations are significant and complex. In the backcross progeny, *L. esculentum* × (*L. esculentum* × *L. hirsutum*), a^+ and sp^+ are less frequent than expected though not significantly so. The segregation of *Wo* and *d* is of particular interest because these two genes are linked.

The crossing over value (genetic observation) between *Wo* and *d* in the F_1 *L. esculentum* × Chillon is lower than that of *L. esculentum* or the F_1 *L. esculentum* × Baños, and the difference may be accounted for by one or more of the following factors: A. Abnormal segregation of the individual genes; B. Selective elimination of recombination gametes; and C. Actual reduction in cytological crossing over or the chiasma frequency.

The first possibility is excluded since both *Wo* and *d* segregate normally. Selective elimination is known in other organisms such as corn, but none has yet been reported in Lycopersicon. The F_1 *L. esculentum* × Chillon has a higher pollen abortion (Table 4) than either of its parents or the F_1 *L. esculentum* × Baños. All the higher pollen abortion in this F_1 however, cannot be attributed to the recombination products of the first linkage group alone, considering that 11 other groups also are present in this hybrid. Besides, we have no positive evidence to show that the aborted pollen is all or almost all of recombination types and not of parental types, since in the parents pollen abortion is seven to eight percent as compared to 14.8 percent in the F_1. The pollen abortion is then, pos-

sibly, caused by the interaction between complementary genes from both species. This hypothesis of interactions between genes of two species becomes more probable since there are also other complementary factors which control the withering phenomenon in the F_1 *L. esculentum* × Baños (SAWANT 1956).

Cytological studies show a distinct reduction in chiasma frequency in the F_1 *L. esculentum* × Chillon (Table 4) per full complement as well as for the second chromosome, on which *Wo* and *d* are located. Therefore, the most likely explanation of the low genetic crossing over value is the reduced chiasma frequency which, in turn, is gene controlled.

Phylogenetic relationships

Finally, taking into consideration the crossability, fertility of F_1's the segregation in F_2's and backcrosses, the crossing over value or linkage in F_1, chiasma number in F_1, and pollen abortion in F_1, it becomes clear that Chillon is less closely related to *L. esculentum* than Baños is. Furthermore, as mentioned previously in the discussion, Baños resembles *L. esculentum* more than Chillon does. Then the question arises, "Do Baños and Chillon belong to the same species?" It is tempting from the above results, to separate them at least as subspecies. It should be mentioned at this point that from 97 flowers of Baños pollinated with Chillon pollen, under field conditions, only three fruits with a few seeds each were obtained. To arrive at a definite conclusion. however, the F_1 of this cross and the segregating generations (provided they can be obtained) must be studied.

SUMMARY

Studies were carried out to determine the relationships between *Lycopersicon esculentum* and the two forms of *L. hirsutum*, the typical form (Chillon) and the form *glabratum* (Baños) by means of crossing relations, sterility of F_1 hybrids, gene segregation, chiasma frequency and crossing over.

In field tests 9.8 percent of *esculentum* flowers set fruit after crossing with Baños and 3.4 percent with Chillon. The same crosses yielded much better in the greenhouse, the crosses of *L. esculentum* × Chillon being 76.6 percent successful —as high as the level of fertility of crosses within *L. esculentum*. Varieties of *L. esculentum* responded differently in the same crosses with *L. hirsutum*. Similarly in these interspecific crosses the number of seeds per fruit varied from 4 to 20, while in the intraspecific it is about 50. The seed set tended to be lower with Chillon than with Baños as pollen parent.

Six genes were introduced into the hybrids from *L. esculentum*, their normal alleles coming from *L. hirsutum*. The assortment of all these genes, however, is not normal. In progenies segregating for *a* and *c* the class *a c* is larger and the class + *c* is smaller than expected on the basis of independent segregation, the deviations being more drastic when Chillon is used as the male parent. Similarly, in the segregation of *sp* and ms_2, the class *sp* + is larger and class + ms_2 is smaller than expected. In this case the large deviations occur in the families in which Baños is used as the pollen parent in the original cross. In the assortment of *Wo*

and *d* the recombination types *Wo* + and + *d* are less frequent since they are linked.

The genetic crossing over between *Wo* and *d* in the F_1 *L. esculentum* × Chillon was significantly lower than the one in F_1 *L. esculentum* × Baños or pure *L. esculentum*. The mean chiasma frequencies for the second chromosome of F_1 *L. esculentum* × Chillon were lower than those in *L. esculentum* or F_1 *L. esculentum* × Baños, showing the correspondence between the cytological and genetic crossing over values. Similarly, the mean chiasma frequencies for the full complement of F_1 *L. esculentum* × Chillon were lower than those in Baños, Chillon, F_1 *L. esculentum* × Baños, or pure *L. esculentum*.

The pollen abortion in F_1 *L. esculentum* × Chillon was higher than that in Baños, Chillon, F_1 *L. esculentum* × Baños or pure *L. esculentum*.

All of these cytological and genetical studies, therefore, confirm the separation of *L. esculentum* and *L. hirsutum* as distinct species. The studies reveal, furthermore, that Baños is more closely related to *L. esculentum* than is Chillon.

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SISTER CHROMATID EXCHANGES IN TRITIUM-LABELED CHROMOSOMES[1]

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By allowing chromosomes to duplicate once in the presence of tritium-labeled thymidine and then following the distribution of the labeled DNA (deoxyribonucleic acid) in subsequent divisions in the absence of labeled precursors, TAYLOR, WOODS and HUGHES (1957) demonstrated that the original DNA is conserved during duplication and distributed in a very precise manner at each cell division. In addition the chromosomes of the broad bean (*Vicia faba*) were shown to be duplexes, i.e., they are composed of two strands before duplication. At the first mitosis after labeling occurs in interphase, all of the daughter chromosomes are labeled. However, the labeled chromosomes do not distribute the labeled DNA equally to their descendants. At the next division after a duplication in the absence of labeled precursors, they regularly produce one labeled daughter chromosome and one unlabeled daughter chromosome. These facts can only be explained if one original, unlabeled strand and one new, labeled strand were received by each of the original labeled chromosomes. At the next division the labeled strand and a newly replicated unlabeled strand are passed on to one daughter chromosome. The unlabeled strand and another newly replicated, unlabeled strand are received by the other daughter. When these second division chromosomes were observed in colchicine-blocked metaphases in which the daughter chromosomes (referred to as sister chromatids before anaphase separation in mitosis) lie somewhat separated except at the centromeres, reciprocal exchange of segments, sister chromatid exchanges, could be observed. For example, a chromatid might be labeled along only part of its length, but in every such instance the other chromatid was labeled in the segment lying opposite the unlabeled segment.

The experiments described below were designed to test the chromosomes of a liliaceous plant for the two-strand property. When the existence of the chromosomal duplex was confirmed and the sister chromatid exchanges proved to be frequent and amenable to analysis, additional material was prepared for a quantitative study of the types and frequency of exchanges. The analysis so far has given relatively little information on the natural frequency of sister chromatid exchanges, but a discovery concerning the structural relationship of the two strands of the duplex has emerged, which is of considerable intrinsic interest in addition to its importance for further studies on chromatid exchanges and crossing over.

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MATERIALS AND METHODS

Roots on bulbs of *Bellevalia romana* were grown in mineral solutions containing 2–3 μg of thymidine-H^3 (specific activity 300–400 $\mu c/\mu$ mole) per ml of solution. The thymidine-H^3 was part of the material prepared by DR. W. L. HUGHES for our original experiments. The specific activity is lower than originally estimated because of the presence of thymidine derivatives produced in the exchange reaction. These derivatives were not detected on the paper chromatograms first used for the separation of the labeled thymidine because they do not absorb ultraviolet light at the same wave lengths as thymidine. However, they have the same or nearly the same Rf value as thymidine in the solvents used. The derivatives were discovered when carrier was added and the thymidine recrystallized (HUGHES, personal communication). In collaboration with DR. W. L. HUGHES of the Brookhaven National Laboratory, Schwarz Laboratories, Inc., Mount Vernon, New York, have prepared and purified thymidine-H^3 of high specific activity, which is now available commercially. Samples of this material have been available for recent experiments. Since the mixture as well as the purified product labels only the chromosomes, the impurities did not seriously interfere in these experiments.

Two procedures are used for labeling the chromosomes and following the distribution of the label in subsequent divisions. All roots were grown at 25±.5°C. After 6–10 hours in the isotope solution roots were either fixed or washed and transferred to isotope free solutions. In one series of experiments designed to observe the division of labeled chromosomes in the absence of colchicine, roots were fixed immediately after removal from the isotope solution and after ten and 18 hours in the solutions free of the isotope. Since labeled anaphases appeared infrequently in these preparations because of the unsynchronized division cycle of the cells, subsequent fixations were made after roots had been in isotope solution six hours, isotope free solution 12 hours and in colchicine for 12–14 hours. The latter preparations yield diploid, colchicine-blocked metaphases (c-metaphases) at the second division after labeling. The chromosomes in these cells are easier to spread and analyze than in the tetraploid cells. However, when analysis of sister chromatid exchanges was made, it became necessary to have all of the chromosomes derived from the original labeled complement in one cell. Therefore, colchicine was used as in the original experiments (TAYLOR *et al.* 1957). In this series of experiments roots were placed in the isotope solution for 6–8 hours and then fixed after ten hours in the colchicine solution. After some preliminary trials colchicine was used at a concentration of 600μg per ml of mineral solution. This concentration is almost completely effective in blocking anaphase and cell division (cytokinesis), but does not prevent reorganization of an interphase nucleus and a second duplication of the chromosomes.

Cells were fixed in acetic-alcohol (3:1), hydrolyzed and stained by the Feulgen procedure. Very much flattened squash preparations were made. The preparations were frozen on dry ice and autoradiographs prepared as described previously (TAYLOR *et al.* 1957; TAYLOR 1956).

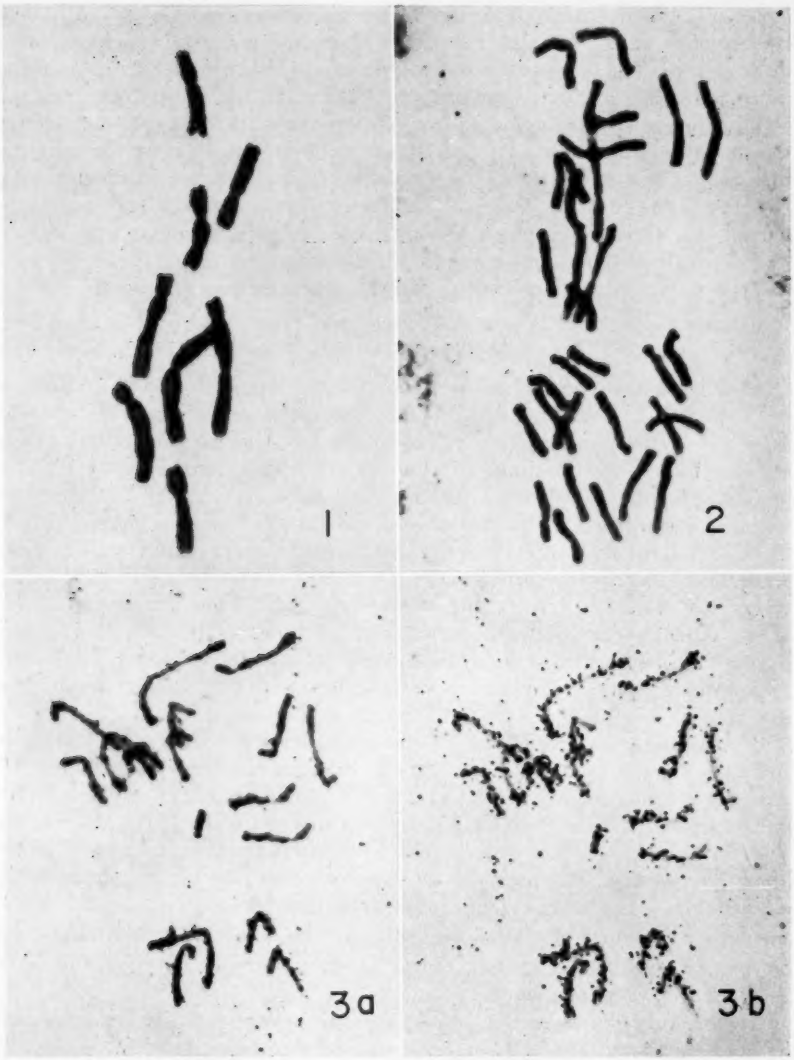

FIGURE 1.—C-metaphase chromosomes of Bellevalia (diploid). × 1650.

FIGURE 2.—Tetraploid c-anaphase: the sister chromatids have separated completely. × 1500. × 1500.

FIGURE 3.—Flattened anaphase at the first division following labeling with tritium-thymidine. × 1150. 3a.—Focal level of chromosomes. 3b.—Focal level of grains.

Bellevalia is advantageous for these experiments because it has only eight large chromosomes in diploid cells (Figure 1). Three morphological types are readily distinguished. The largest chromosome, designated chromosome I, has a median centromere. The medium sized one, chromosome II, has arms of very unequal length and is easily distinguished from chromosomes III and IV. Unfortunately, the smallest chromosomes, III and IV, are so nearly alike morphologically that they cannot be distinguished from each other. Therefore, they will be treated as one class in the analyses. The sister chromatids lie together like those in Figure 1 during most of the c-metaphase. However, for a short period in late c-metaphase, they separate as shown in Figure 4a. Finally, the sister chromatids move apart and sisters cannot regularly be recognized by their position (Figure 2).

EXPERIMENTAL RESULTS

After ten hours in the isotope solution many interphase nuclei, and nearly all of the prophase, metaphase and anaphase chromosomes were uniformly labeled from end to end in so far as autoradiographs allow determination of this characteristic (Figure 3). Certainly all parts of every chromosome in the flattened anaphase figures were labeled. Although unlabeled sectors might escape notice in some cases with grain densities as low as those shown in the photograph (Figure 3), many of the figures had two to three times the grain density shown. These were unsatisfactory for grain counting, but they showed that unlabeled sectors do not exist at the first division following incorporation of the isotope. At least they do not under the conditions of these experiments, in which the isotope was present during the whole period of duplication of the chromosomes observed. The c-metaphases in preparations fixed after 8 hours in the isotope and 10 hours in colchicine also showed all chromatids labeled throughout their length.

By counting grains over pairs of daughter chromosomes similar to those shown in Figure 3b, data on the equality of the distribution of isotope was obtained. The data are recorded in two ways in Figure 7. First the number of grains over a chromosome in a right hand anaphase group was plotted along the abscissa, and then the number of grains over its sister in the other anaphase group was plotted along the ordinate. The points at the intersection of coordinates plotted in this way, fall on a line with a slope of one when the counts are equal. The experimentally determined points fall near the line with no significant deviations. The two counts showing the greatest deviation when tested by chi square for a 1:1 ratio have a P value between .30–.40.

Another way of plotting the data is shown in the right hand corner of the same graph (Figure 7). The ratio of isotope in each two daughter chromosomes is obtained by dividing the number of grains over the one with the higher number by the number of grains over the one with the lower number. Then the deviations of these ratios from unity are plotted as a frequency distribution. The frequency polygon appears to fit a normal curve, which indicates that the variations are random, and therefore, the distribution of label may be considered equal.

However, a more convincing test, for the precision of the segregation of the original and the newly formed DNA, can be made at the second division. Fortunately, the pool of labeled precursors is small and is depleted in a very short time after the roots are removed from the labeled solutions. To obtain cells with chromosomes that had replicated once after labeling, roots were allowed to grow for 6–8 hours in the isotope solution, then 10 hours in mineral solution free of isotope and finally 12–14 hours in colchicine solution to accumulate a number of c-metaphases. Some of these diploid c-metaphases contained labeled chromosomes. The chromosomes had one chromatid labeled and one free of label at any level along the chromosome (Figure 4). However, one of the striking features was the high frequency of sister-chromatid exchanges. Because of the small number of chromosomes these diploid metaphases are ideal for determining the number of sister chromatid exchanges. For example, one chromosome I (Figure 4) shows no exchanges while the other has three. One chromosome II near the center of the group has one exchange and the other chromosome II at the lower right, which is lying under the chromosome I without exchanges, has two exchanges. However, this may not be evident from the photograph. One of the chromosomes III–IV has no exchanges (near the center of the group); two have one exchange and the one in the left center has two exchanges.

Although sister chromatid exchanges are more easily analyzed in diploid cells like the one shown (Figure 4) than in tetraploid cells, the diploid cells do not give all of the information on the frequency and types of chromosome exchanges. The tetraploid cells at the second division after labeling contain all of the chromosomes derived from the original labeled complement and allow a more complete analysis of the origin of the exchanges.

One of the striking features of these labeled tetraploid cells was the frequency with which twin exchanges occurred in homologous chromosomes (Figures 5 and 6). Twin exchanges are defined as sister chromatid exchanges at the same locus in two separate chromosomes at the second c-metaphase. Since the two chromosomes have been separated since the first c-metaphase the twinning must reflect some event that occurred before the separation of the chromosomes at the first division. With this in mind the number of twin and single exchanges were counted. The frequency of each type is given in Table 1. Since the frequency of single exchanges was relatively low the classification of the two types was quite reliable especially in the two largest chromosomes. In the smallest chromosomes, two single exchanges may occasionally occur near enough the same locus in two of the eight chromosomes to be mistaken for a twin. Confidence that exchanges are really at the same loci in two separate chromosomes is gained by observation of instances in which two sets of twin exchanges in one pair of chromosomes match exactly (Figure 8, chromosomes II).

Exchanges were recorded (Table 1) only when all four of the homologous chromosomes in a particular cell could be analyzed. This meant that a considerable number of cells at the proper stage of colchicine treatment, which were sufficiently labeled to produce autoradiographs, could not be used because some

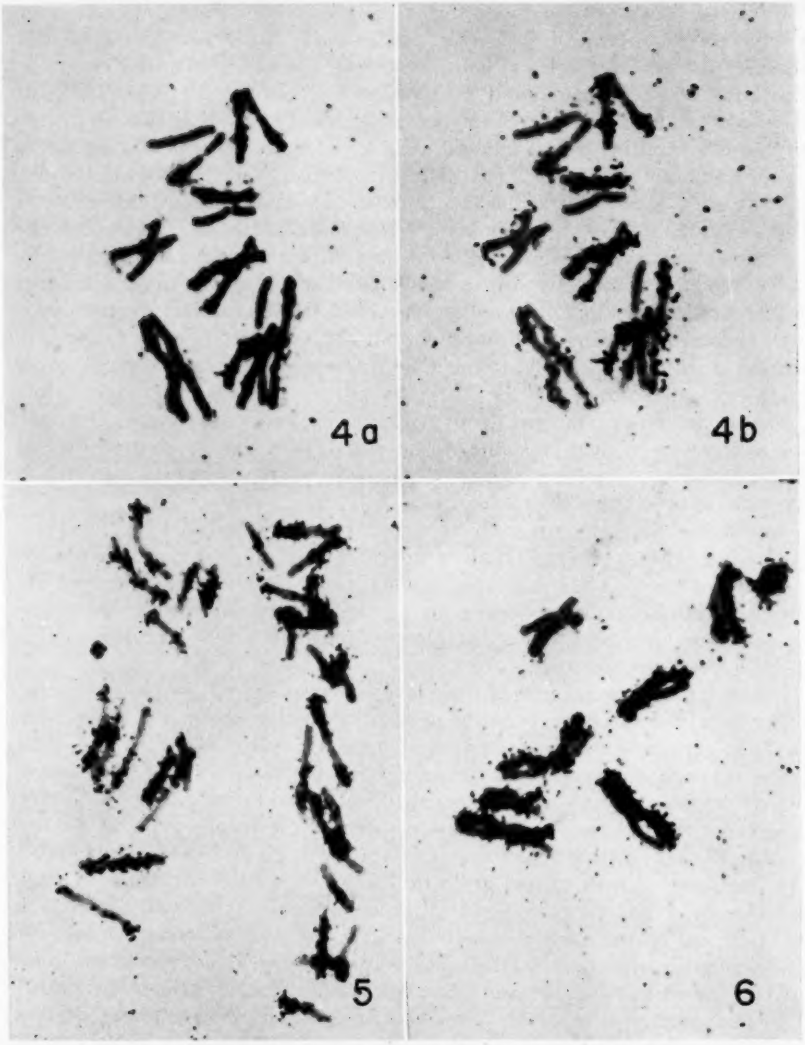

FIGURE 4.—C-metaphase at the second division after labeling (diploid cell). × 1650. 4a—Focal level of chromosomes. 4b.—Focal level of grains.

FIGURE 5a.—C-anaphase at the second division after labeling (tetraploid cell) when the sister chromatids have completely separated. × 1500.

FIGURE 6a.—Part of the complement of a tetraploid c-metaphase at the second division after labeling before chromatids separate. × 1500.

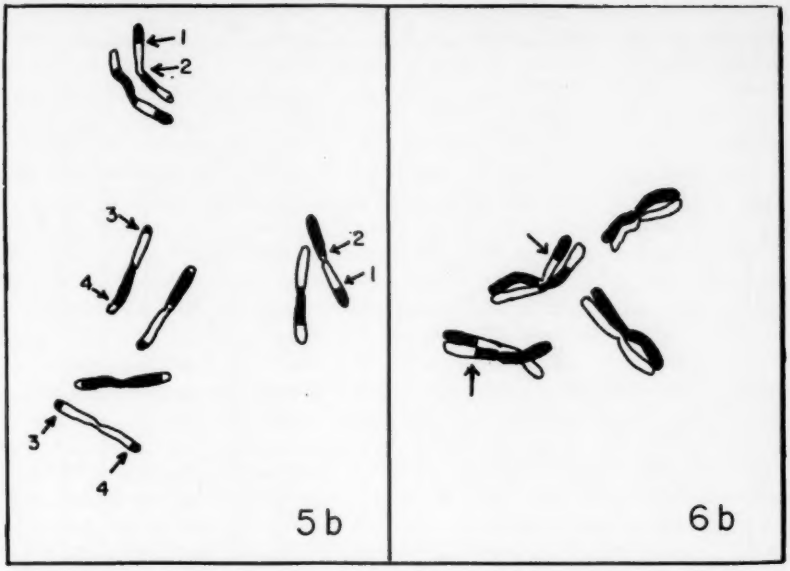

FIGURE 5b.—Drawing of chromosomes I from FIGURE 5a showing exchanges; twins indicated by arrows (black regions represent labeled portions).

FIGURE 6b.—Drawing of chromosome I from FIGURE 6a to show the position of exchanges; twin exchanges indicated by arrows.

TABLE 1

The frequency and types of exchanges observed at the second c-metaphase in root tip cells of Bellevalia

Chromosome	Number of chromosomes examined (chromatid pairs)	Number of twin exchanges	Number of single exchanges	Mean frequency of exchanges per chromosome	
				1st interphase (twins)	2nd interphase (singles)
I	72 (18 cells)	36	15	1.00	0.21
II	52 (13 cells)	17	4	0.66	0.08
III, IV	80 (10 cells)	28	11	0.70	0.14
Totals	204	81	30		

of the four chromosomes were lying under or over some others of the complement. Some cells were analyzed after they were at c-metaphase long enough for sister chromatids to become separated and sometimes displaced from their sisters (Figure 5). By the pattern of exchanges and morphological features of the chromosomes a reconstruction of the original relationships could sometimes be made, especially if part of the sister chromatids were still adjacent to each other. Figure 8 shows the reconstruction of the complement from such a cell.

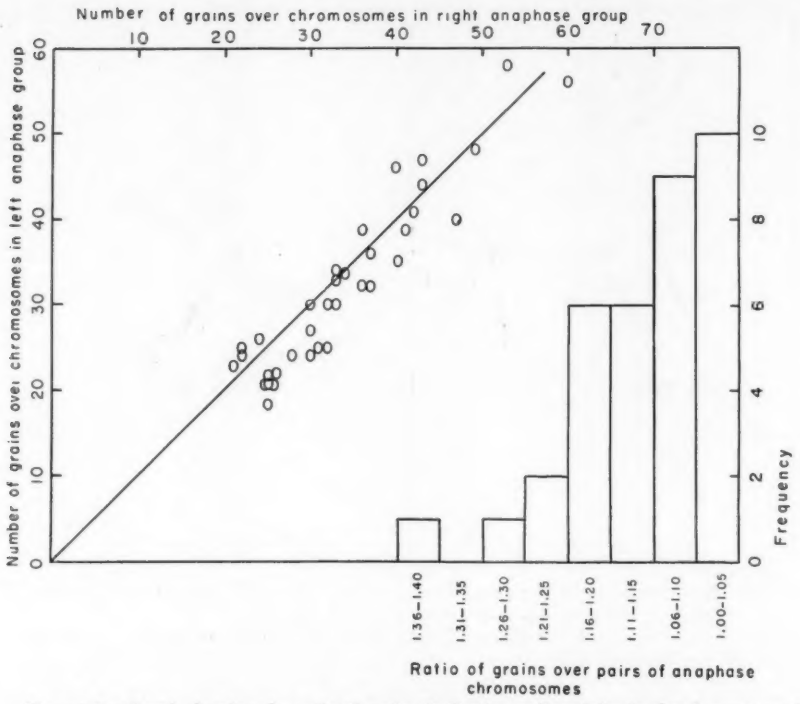

FIGURE 7.—Graph showing the ratio of grains (reflecting radioactivity in the chromosomes) over sister chromosomes in anaphase at the first division after labeling.

Twin exchanges did not occur in the diploid c-metaphases (Figure 4). Their failure to appear in these cells shows that the breaks are not localized by some mechanism which would simulate twin exchanges, and therefore, the observation adds to the confidence of the interpretation of twins. Among 72 chromosomes I examined (18 tetraploid cells), there were 36 twin exchanges (72 chromosomes with exchanges) and 15 single exchanges (Table 1). For chromosome II, 17 twins and four singles were observed among 52 chromosomes (13 tetraploid cells). Altogether among 204 chromosomes examined, there were 81 twins to 30 single exchanges. Interpretation of the frequency of exchanges per chromosome will be deferred until a consideration of the types of exchanges possible is made (see discussion).

Distribution of the isotope at the second division was regularly all or nothing as previously stated and nearly all breaks appeared to be clean cut in so far as resolution will allow determination of this feature. However, a few exceptions noted are worth recording. Among 204 chromosomes analyzed for exchanges at

the second division, two instances of apparently equal distribution of the isotope to both chromatids from the locus of an exchange to the end of the chromosome were noted. One instance of equal distribution in an interstitial segment between two exchanges was seen also. One chromosome in a cell not recorded in Table 1, because not enough of the chromosomes were lying separately, showed equal distribution for the whole length of one chromosome. All others in the complement that could be analyzed showed the typical all or nothing segregation. In all of the instances the regions with equal distribution show the isotope to be about equal in amount in the two chromatids to that in a single chromatid of the remainder of the complement. In spite of the above mentioned discrepancies no instance was found in which opposite segments of both chromatids were unlabeled. This confirms the observation that unlabeled segments do not occur at the first division, for if they did the result at the second division should be that both sister chromatids would be free of label in some segments of the chromosomes.

Although no effort was made to determine quantitatively the dose of the endogenous radiation from the tritium, it was enough to produce aberrations. A fragment may be seen at anaphase of the first division in Figure 3. In addition among about fifty metaphases examined for sister chromatid exchanges, four interchromosomal exchanges were seen. All of these were of the chromosome type, i.e., the rearrangements involved both chromatids in each chromosome. Of course, they could have been derived from the duplication of chromosomes in which chromatid exchanges had occurred at the first division.

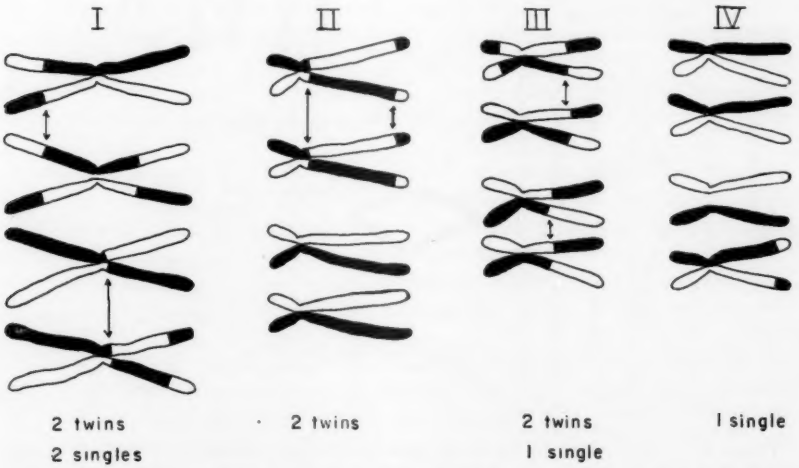

FIGURE 8.—Diagramatic reconstruction of the chromosome complement from a tetraploid c-metaphase at the second division after labeling to show the position of exchanges; twins indicated by arrows.

DISCUSSION

Since each chromatid is a duplex, the possibility of an exchange involving only one strand of each duplex should be considered. If an exchange occurred between a labeled strand of one duplex and an unlabeled strand of the other duplex, anaphase chromosomes at the first anaphase after labeling occurred would contain unlabeled segments (Figure 9a). Since the chromosomes were uniformly labeled

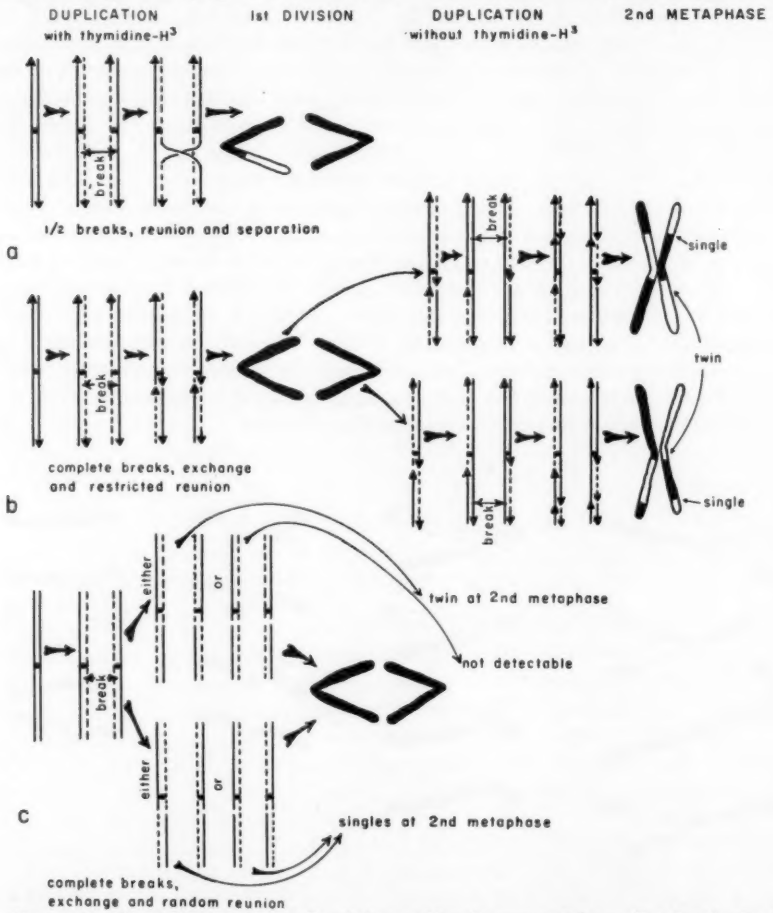

FIGURE 9.—Chart showing the results of chromatid exchanges and the predicted ratio of twin and single exchanges. (a). The predicted results of one-half chromatid exchanges which were not observed. (b). The results of exchanges when reunion is restricted by a difference between the two strands of the chromatids. (c). The results of exchanges when reunion is unrestricted and rejoining of strands occurs at random. Labeled units are shown as dashed lines. The difference between strands is represented as a directional sense and is indicated by arrows.

at this stage, we conclude that this type of exchange did not occur. Instead all exchanges observed resulted from the breakage of whole chromatids, i.e., both strands of each duplex broke at the same or nearly the same locus.

Half-chromatid exchanges have been described a number of times following irradiation of cells since they were first reported by NEBEL (1936) and by SWANSON (1947). They are seen as anaphase bridges without accompanying fragments. Photographs published by CROUSE (1954) and by LACOUR and RUTISHAUSER (1954) are particularly clear. However, aside from these observations, there is no direct experimental evidence concerning the mechanism of the origin and disposition of these bridges. ÖSTERGREN and WAKONIG (1954) obtained negative evidence that the half chromatid exchanges did not occur, i.e., that the "stickiness" observed at anaphase involved only the matrix of the chromosomes. This conclusion was based on the failure of the half exchanges to appear as whole chromatid exchanges at the next division when roots were grown in colchicine to prevent the breakage of the connections at anaphase. Neither their evidence nor the observation of labeled chromosomes reported here demonstrates that the half exchanges do not occur. These experiments only show that they do not persist either after a normal anaphase or after a c-mitosis. They could be first events in whole chromatid exchanges. Their occurrence might produce an unstable condition which either undergoes restitution or results in breakage and exchange of whole chromatids at some time before the second c-metaphase.

The occurrence of the twin exchanges in the tetraploid c-metaphases and their absence in the diploid c-metaphases indicate that they are due to events occurring before the separation of the sister chromatids at the first division after labeling. This leads us to a consideration of the types of exchanges that can occur between duplexes (chromatids) each of which has one labeled strand and one unlabeled strand. We will discuss two possibilities: (a) that reunion is restricted by a difference in the two strands of the duplex, and (b) that reunion is unrestricted (Figure 9).

If the two strands of the duplex are different in some way, for example in directional sense analogous to the two strands of the WATSON and CRICK (1953) model of DNA, the types of reunion would be restricted (Figure 9b). If we suppose the two strands have opposite sense, and that only strands of like sense can rejoin when exchange occurs, a labeled and an unlabeled strand will regularly rejoin in each chromatid. At the first division both daughter chromosomes will appear labeled throughout their length. After another duplication in the absence of labeled precursor the exchange will be revealed. Each two c-metaphase chromosomes, descended from a chromosome with the type of exchange described above, will have sister chromatid exchanges at the same locus, i.e., twin exchanges will appear at the second division. Each of these second division chromosomes may also contain single exchanges which can occur after the second duplication. Since at this stage only one of the chromatids has a labeled strand at any level, each exchange will become visible at the division immediately following, i.e., the second c-metaphase. If we assume that the same probability for breakage and

exchange exists at both the first and second divisions, we predict a ratio of one twin to two singles according to the following reasoning. Let the probability of a break in both chromatids of a chromosome during the first interphase when labeling occurs be p. For the second interphase let the frequency be q and assume that the same chance for exchange or restitution of the breaks occurs in both interphases. Further, assume either (1) the rejoins are restricted by a difference in the two strands and only like strands can rejoin, or (2) that the rejoining is not restricted and the four ways to rejoin are equally possible (Figure 9c). When p and q are equal the ratio expected for twins to singles with restricted rejoining is 1:2, and for unrestricted rejoining the ratio is 1:10. The latter ratio is the minimum figure for if unrestricted rejoining could occur a break in a single chromatid with rotation of 180° and rejoining at any time before the second interphase duplication would produce single exchanges. Their frequency cannot be predicted, but if they could occur the ratio of twins to singles would be a fraction considerably smaller than 1:10. When q equals zero, i.e., no exchanges occur in the second interphase, the ratio would be 1:0 for restricted rejoining and 1:2 for unrestricted rejoining. An examination of the data in Table 1 shows that both twins and single exchanges were observed at the second c-metaphase in tetraploid cells, i.e., when all of the descendants of the original complement of labeled chromosomes could be examined. However, the number of singles is considerably less than predicted on either the restricted or unrestricted rejoining hypothesis when p equals q, i.e., when the frequency of breakage and exchange is equal in the two interphases. However, the hypothesis of unrestricted rejoining can be eliminated because the frequency of twins is several times higher than would be predicted even when no exchanges occurred in the second interphase. Unrestricted rejoining would produce a ratio of one twin to two singles while the observed ratio was 81 to 30. The conclusion must be that rejoining is restricted and the frequency of exchanges in the second interphase is lower than in the first interphase.

With the knowledge that the rejoining is restricted and that all exchanges in the first interphase produce twins, a calculation of the frequency of exchanges at the two divisions can be made (Table 1). The frequency of sister chromatid exchanges for chromosome I is 1.0 in the first cycle and 0.21 in the second cycle. For the smaller chromosomes the frequency is lower, 0.66–.70 exchange per chromosome in the first cycle. The lengths of the chromosomes are in the ratio 100:75:65 for chromosomes I, II, and III–IV, respectively. Therefore, the frequency of exchanges is very nearly proportional to length.

The lower frequency of exchanges in the second interphase can be correlated with two differences in the two cycles. The amount of endogenous radiation is at least reduced by one half at the second interphase. Perhaps the difference is even greater if the thymidine derivatives enter the cell but are not utilized. By the second interphase they might be transported to other parts of the plant or lost into the culture solution. The other difference is that the second interphase occurs in the presence of colchicine while most of the first interphase is completed before the roots are placed in colchicine. BRUMFIELD (1943) reported that colchi-

cine reduced by two thirds the chromatid aberrations induced by X-rays. It appears possible that colchicine might also reduce any naturally occurring sister chromatid exchanges as well as radiation induced ones. Although it is not possible to predict what fraction of the exchanges, if any, occur naturally, the conclusion with respect to restricted reunion stands. Even if all of the exchanges were occurring in the first interphase the number of singles is far less than would be predicted on the basis of unrestricted reunion.

Therefore the interesting and significant conclusions are (1) that all breaks are four strand breaks and reunions and (2) that the two strands of the chromosomal duplex are not alike. The doubleness is almost certainly a fundamental property of all chromosomes. No matter how many DNA and protein chains may be present in a chromosome, these must be organized into two units that are opposite in some sense. This oppositeness could be either a mirror image difference between the two strands or a difference in directional sense with or without complementarity. However, since the two units of the duplex fit together so intimately that the new and old ones rarely if ever separate at the division immediately following duplication, they are likely to be complementary in the chemical sense.

The two polynucleotide strands of the WATSON-CRICK model of DNA are different in directional sense and therefore are analogous to the chromosome in this respect. A chromosome thus might be a single double helix. However, the possibility that the chromosomal duplex is composed of *two* WATSON-CRICK double helices is certainly eliminated, for these would be identical.

To obviate the problems of coiling such a long strand (over one meter in large chromosomes) of DNA into a chromosome and of unwinding the stands during duplication, TAYLOR (1957) has proposed that the chromosome is an array of DNA double helices attached to a central core. The core is visualized as a double ribbon with DNA double helices attached along the edges so that each helix has one polynucleotide chain attached to one ribbon and the other chain of the same helix attached to the other ribbon, perhaps by a terminal phosphate group. Each ribbon with its attached DNA chains, possibly on both edges to make it symmetrical, would comprise one unit of the duplex. The duplex chromosome would separate into its components during duplication. Another ribbon would be built along each separating ribbon and the DNA double helices would begin to pull apart, with the separation from each other starting at their points of attachment to the ribbons. With the unattached end free to rotate and with rotation of strands possible at single bonds along the polynucleotide chains in the separating regions, replication could proceed according to the WATSON and CRICK scheme. All new polynucleotide chains would be attached to the new ribbons and would segregate as units in future duplications of the chromosomes, while the sister chromatid exchanges would represent breaks and exchanges along the axis.

At what stage in the cell cycle do the sister chromatid exchanges occur? Are they formed as a part of the duplication process and are they mechanically comparable to crossovers? The analysis indicates that the exchanges occur either during duplication or between the time of duplication and separation of daughter

chromosomes. Since the two strands of the chromosome are different, no strand exchanges can occur between the time of separation and the time of the next duplication. Duplication then produces strands that can exchange with the original ones, although the two new strands cannot exchange with each other; they are unlike. As mentioned earlier exchanges between two of the four strands might occur, but this requires a breakage of at least one of the original strands. Such half exchanges might be more likely during duplication, but nothing observed so far would limit exchanges to the period of duplication. Half exchanges if they occur at all do not persist until the second c-metaphase. Mechanically the exchanges may be comparable to crossing over, but the evidence is not compelling. Any study of crossing over must, of course, take into account the difference in the two strands of the chromosome.

SUMMARY

By following the distribution of tritium-labeled DNA in chromosomes of Bellevalia, they are shown to be two-stranded before duplication. Both chromatids of each chromosome are labeled at the first metaphase or anaphase after incorporation of tritium-labeled thymidine. After another duplication in the absence of labeled precursors each labeled chromatid (chromosome now) yields one labeled daughter and one free of label at any level along the length. Sister chromatid exchanges were frequent, but half-chromatid exchanges, i.e., exchanges between one strand in each of two chromatids, were not observed. Sister chromatid exchanges were frequently observed to occur as pairs or twins, i.e., exchanges occur at the same locus in two of the four homologous chromosomes in the second c-metaphase after labeling. Among 204 second metaphase chromosomes examined there were 81 twin exchanges and 30 single exchanges. This frequency can be explained only on the hypothesis that the two strands of the chromosome are unlike, i.e., are not free to reunite at random. Therefore, the chromosome has two features in common with the WATSON-CRICK model of DNA. It has two strands and the strands are different in some structural feature that restricts reunion to like strands when chromatid exchanges occur.

ACKNOWLEDGMENT

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THE GENETICS OF SCHIZOPHYLLUM COMMUNE*

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DURING the course of an investigation on sexuality and related phenomena in the common wood-rotting mushroom, *Schizophyllum commune*, a continuing search has been maintained for appropriate genetic markers for use in the work. A wide variety of morphologically aberrant and biochemically deficient strains has accordingly been tested for single locus segregation and for possible linkage with the factors determining mating behavior and with each other. The current report presents a brief description of the organism, the materials and methods employed, and the genetic results of the study to the present time.

General characteristics, life cycle, and sexuality of Schizophyllum

The life cycle, basic pattern of sexuality, and the mode of sexual reproduction in Schizophyllum are typical of the group of higher fungi comprising the bracket fungi, pore fungi, and gilled mushrooms, a group collectively termed the Hymenomycetes. The Hymenomycetes include among its members the most important of all wood rotting organisms, many plant pathogens, particularly of forest trees, and numerous species that provide food for a wide array of animals. Considerable use has been made of various species of the group in biological research, but such work has dealt predominantly with only two aspects of their biological activities: the basic pattern of sexuality peculiar to the group and the decay of wood and wood products. Although the members of the group are the most conspicuous and superficially the best known of all fungi, their developmental history is sketchily known to most biologists. A brief description of Schizophyllum and of its life history is accordingly necessary to the present account. (For a fuller account of life history and sexuality, see BULLER [1941], WHITEHOUSE [1949], and RAPER [1953].)

Schizophyllum is a gilled fungus, a mushroom, but the taxonomic position of the genus has long been in doubt; early authors included it in the Agaricales, the gilled fungi, but many recent authors assign it to the Polyporales, the bracket and pore fungi (GAÜMAN and DODGE 1928; HEIM 1948). The genus comprises several species (LINDER 1933), of which *S. commune* is the most common. It is widely distributed throughout temperate and tropic zones and commonly occurs as a weak parasite or saprophyte on a wide range of woody plants and occasionally on herbaceous plants.

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The genus Schizophyllum differs from all other gilled forms in that its gills are made up of two flat plates which curl away from each other under conditions of desiccation. It also differs from the typical mushroom in the peripheral and indeterminate growth of its fruit body. The fruit body primordium early develops a tiny concave pit lined with sporulating elements. Growth at the periphery of this pit continually enlarges the sporulating surface; the area of the latter becomes further extended by the development on the base of the pit of radial ridges which form the double plated gills. The well known "button" stage of the typical mushroom is lacking.

Basidiospores are produced in profusion on the under surface of the fruiting body. Each spore germinates under proper conditions by apical hyphal growth from one end or from both ends of the swollen spore and produces a septate, homokaryotic mycelium constituted of mononucleate cells. This mycelium is capable of indefinite vegetative growth.

Contiguous homokaryotic mycelia establish interconnecting hyphal bridges through which nuclei of the two mycelia become associated. The pattern of subsequent development, however, depends upon a precise discrimination based upon a complex incompatibility differentiation. If the two mated mycelia are compatible, there follows a sexual interaction with the reciprocal "fertilization" of each mate by nuclei from the other.

The sexual reaction consists of two basic processes subsequent to the entry of compatible nuclei *via* hyphal anastomoses into a pre-existing mycelium: (a) the migration and the multiplication of these nuclei throughout the entire mycelium, and (b) the establishment of an indefinite number of pairs of migrant and resident nuclei, each pair termed a dikaryon. The dikaryotic mycelium thus initiated is capable of indefinite vegetative growth, during which the members of the dikaryon of each terminal cell divide synchronously (conjugate division), as a result of which each daughter cell contains a pair of compatible nuclei. In conjugate division, one nucleus divides along the axis of the original cell while one daughter of the other nucleus passes to the forming subterminal cell through a lateral hook (the clamp connection—an invariable diagnostic feature of dikaryotic mycelia in Schizophyllum). Neither homokaryotic nor dikaryotic mycelia produce asexual reproductive spores.

The dikaryotic mycelium eventually produces the fruiting body, in which occur nuclear fusion and meiosis, the final stages of the sexual cycle. Extensive proliferation of single scattered vegetative cells leads to the differentiation of fruit body primordia which further develop into the sporulating fruits at the expense of the surrounding mycelium. A columnar layer of cells, located on the lower surface of the expanded cap of the fruit, is made up of elongate and club-shaped basidia. Within each basidium the two nuclei of the dikaryon fuse, and the fusion nucleus soon thereafter divides meiotically to yield four haploid nuclei. These four nuclei then migrate singly into four apical basidial outgrowths which develop into a tetrad of exogenous basidiospores.

S. commune is heterothallic. The pattern of mating behavior between homo-

karyotic mycelia is based upon a bifactorial incompatibility mechanism, in which each of two incompatibility factors, *A* and *B*, occurs in an extensive series of alternate states, originally interpreted by KNIEP (1922) as multiple alleles (see below). The *mating type* of a homokaryotic mycelium is determined by the specific *A* factor and specific *B* factor present, and sexual interaction to establish the dikaryotic mycelium occurs only between mycelia that are heterozygous for both factors. Each dikaryotic mycelium accordingly contains two dissimilar *A* and two dissimilar *B* factors, and at meiosis, following the brief diploid phase, the independent assortment of the incompatibility factors results in the production of spores of four distinct mating types in equal frequency. The term, *tetrapolar sexuality*, was applied to this pattern of sexual behavior by KNIEP (1920) to denote the occurrence of spores of four types from each fruiting body.

The life cycle and sexuality of *S. commune*, as applied to a single fruiting dikaryotic mycelium, are schematically represented in Figure 1.

Paralleling the establishment of the dikaryotic phase in the normal life cycle but of unassayed significance in the total biology of the organism are a number of additional mycelial interactions. Matings of homokaryotic mycelia in combinations of incompatibility factors other than the double heterozygote yield three distinct types of sexually sterile heterokaryons. These interactions and their heterokaryotic products are germane to the present account only to the extent that numerous mutant strains have been derived from one of them, the common *A* heterokaryon.

MATERIALS AND METHODS

The materials used in this study have been derived largely from four strains of the *Argonne* stock, originally isolated and studied by PAPAZIAN (1950, 1951). A number of additional strains originating from other stocks have also been used, but to a lesser extent. The stocks from which these strains were derived are as follows: *Overholts* I, a dikaryotic stock maintained since 1932 in the culture collection of the Forest Products Laboratory, U. S. D. A., Beltsville, Maryland; *Big Sur*, collected in 1951 at Big Sur, California; *Fresh Pond*, collected in 1955 in Cambridge, Massachusetts; and *Walden III*, collected in 1955 in Concord, Massachusetts.

A total of about 35 characters have been examined in random samples of monosporous progeny of some 70 matings which ranged from 2- to 7-point crosses. The characters dealt with here constitute two distinct categories:

Incompatibility factors—: The homokaryotic strains employed in the crosses have carried incompatibility factors in numerous combinations of the following: A^1, A^2, A^9, A^{10}, A^{15}, A^{16}, A^{41}, A^{35}, *and* $A^{36} \times B^1$, B^2, B^9, B^{10}, B^{15}, B^{16} and B^{41}. The superscripts identify individual specific factors collected from nature (in the order of their acquisition) and maintained in our collection.

Morphological and biochemical aberrations: All other genetic characters that we have been able to use represent distinct departures from the wild type, either

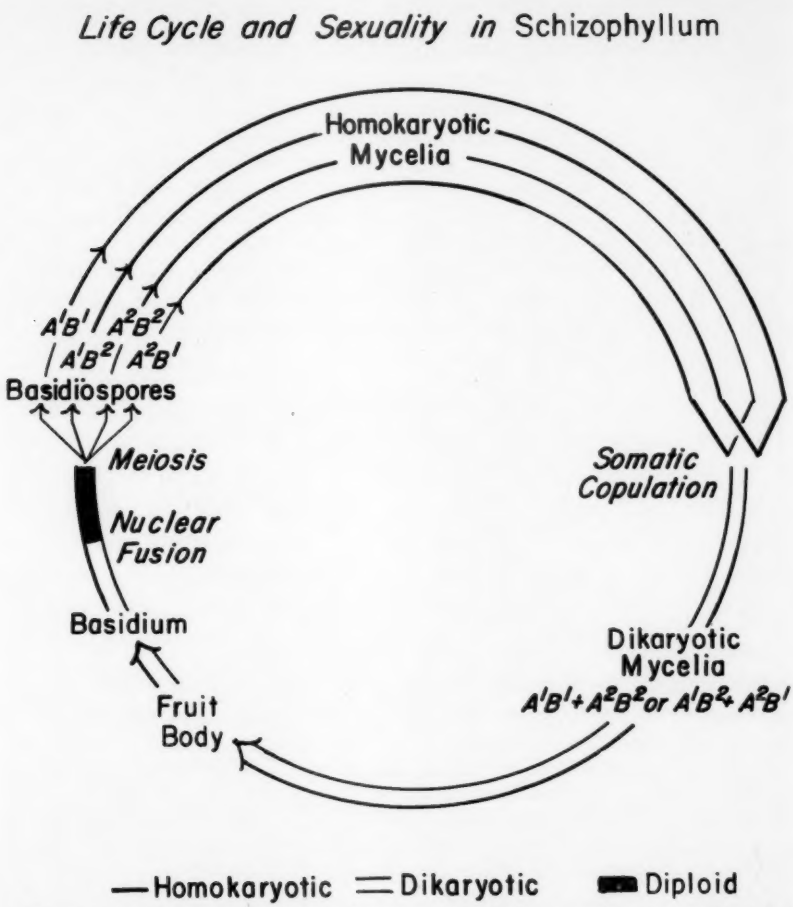


FIGURE 1.—Diagram of life cycle and pattern of sexuality as applied to a single stock of Schizophyllum, a typical tetrapolar Hymenomycete.

as morphological aberrations or as growth requirement deficiencies. These mutant characters originated under four different circumstances:

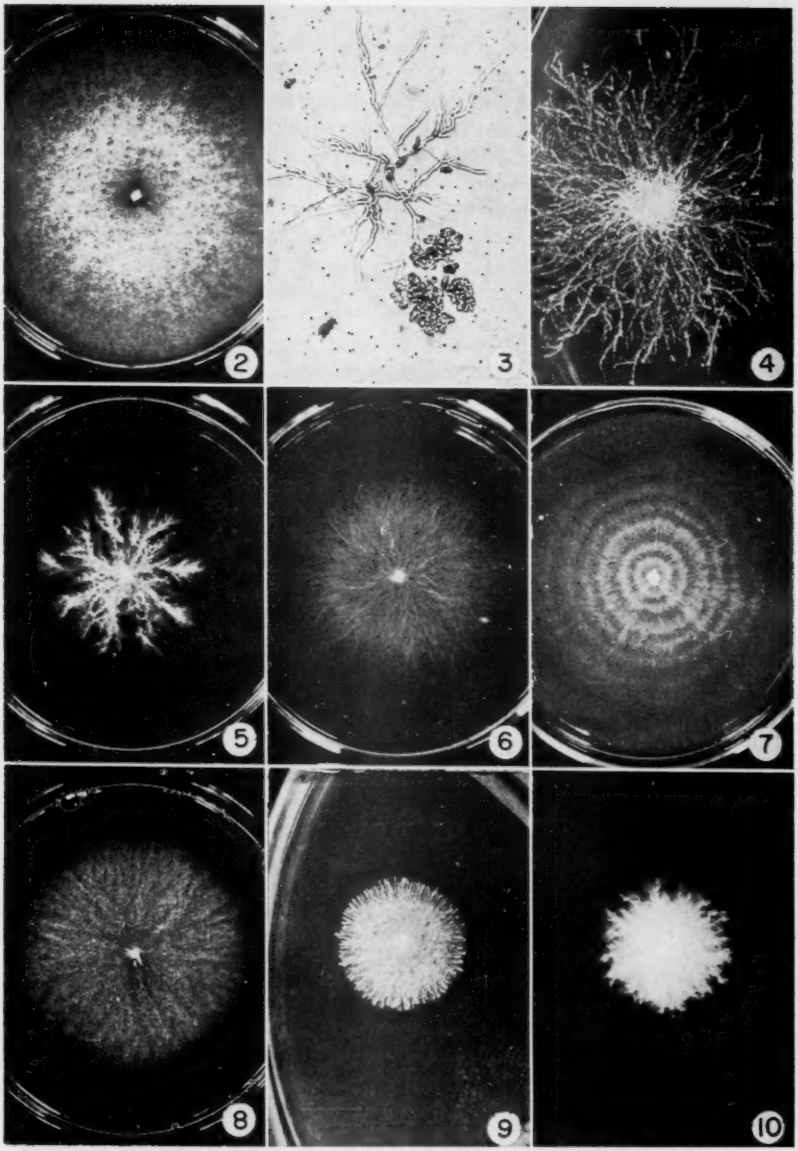
1. Ultraviolet irradiation of macerated homokaryotic mycelia yielded numerous biochemically deficient and morphologically aberrant strains. The nutritionally deficient strains included *uracilless*, *niacinless-1*, *niacinless-2*, *argininеless-1*, *argininеless-2*, *cholineless*, *riboflavinless*, *biotinless*, *phenylalanineless*, *guanine-hypoxanthineless*, *adenineless*, and *hypoxanthineless*. The morphological deviants varied widely in habit and growth rate; of these, only three are included here and are designated *UV-morph I–III*.

2. Isolation of micromycelia from common *A* heterokaryons provided a number of morphologically aberrant strains, termed *het-mutants: streak*, *fir*, *dwarf*, *fluff*, *puff-1*, *puff-2*, *thin*, *feather*, *bubble*, and *early blue*. These mutant types regularly come to visible expression in the sexually infertile, common *A* heterokaryon in the absence of any treatment with mutagenic agents (RAPER, SAN ANTONIO, and MILES, in press). Two or more isolates of independent origins of each of five of the het-mutants, streak, fir, feather, puff, and early-blue, were used in the work. In each of four cases, the results of crosses with different isolates were indistinguishable; in the fifth case, puff, one isolate consistently behaved differently from the other—hence the distinction, puff-1 *vs.* puff-2. In the absence of such differentiating evidence, different isolates of the same mutant phenotype are treated as probably representing distinct mutations at the same locus. Representative het-mutants, puff, streak, early-blue, fir, and feather, are shown in Figures 4, 6, 8, 9 and 10.

3. The Overholts-I stock yielded seven morphological aberrations, *late blue-1*, *late blue-2*, *pigmy*, *concentric*, *felty*, *plumy*, and *restricted*, and a single *unidentified* nutritional *deficiency*. It has been found in the present work that genetically balanced dikaryotic mycelia accumulate a remarkable burden of spontaneous, recessive mutations when maintained in culture over long periods (MILES, unpublished). These mutant characters may be recovered in the progeny of outcrosses between wild type and either (a) the progeny of the dikaryotic mycelium or (b) the original homokaryotic components of the dikaryon (MILES and RAPER 1956). Two mutants segregated from the Overholts I stock, *felty* and *concentric*, are shown in Figures 5 and 7.

4. Occasional morphological and biochemical mutants have been recovered among the progeny of various stocks freshly collected from nature. Only one of these, thiamineless, is included in the current account.

A detailed account of the morphological, biochemical and physiological characteristics of these mutants is in preparation and will be published elsewhere. Although in general the detailed characterization of the mutants is not essential to present considerations, two points require brief explanation. (a) *S. commune* has been described by numerous authors as lacking the ability to synthesize the pyrimidine moiety of thiamine (SCHOPFER and BLUMER 1940; ROBBINS 1938). We have found the pyrimidine deficiency to be quantitatively extremely variable, and we have routinely included thiamine in the minimal medium. Thiamineless requires relatively massive supplementation with thiamine and differs conspicuously from wild type behavior in being unable to grow on our "complete" medium while being indistinguishable from wild type on our "minimal." (b) A majority of the morphological mutants, including most of the UV-morphs and all but three of the het-mutants, puff, bubble, and early-blue, mate unilaterally. In compatible matings, strains carrying any of these aberrations cannot act as receptors of fertilizing nuclei; they can only provide fertilizing nuclei for their mates, unless, of course, these are similarily handicapped. As a consequence there is an effective barrier against dihybrid crosses between the unilaterally mating phenotypes. This barrier has been breached in only a single cross, feather × thin



FIGURES 2–10.—Morphological characteristics of wild type and mutant homokaryotic mycelia of Schizophyllum. FIGURE 2.—Wild type, 9 days, × ½. FIGURE 3.—Germinating spores of wild type (above) and puff, 48 hours, × 100. FIGURE 4.—Puff, 14 days, × 1½. FIGURE 5.—Felty, 9 days, × ½. FIGURE 6.—Streak, 9 days, × ½. FIGURE 7.—Concentric, 9 days, × ½. FIGURE 8.—Blue, 9 days, × ½. FIGURE 9.—Fir, 14 days, × 1½. FIGURE 10.—Feather, 14 days, × 1½.

(Table 4). This was achieved by heavy seeding of minimal medium with macerates of A^1B^2-thin-uracilless and A^2B^1-feather-argininеless. The few resulting dikaryotic mycelia indicated that the cause of unilateral mating lay in a failure of nuclear migration rather than in failure to form hyphal anastomoses.

Routine cultures and matings have been made on a complete medium consisting of 20 gm dextrose, 2 gm peptone, 0.46 gm KH_2PO_4, 1.0 gm K_2HPO_4, 0.5 gm $MgSO_4$, and 20 gm Bacto-peptone per liter of distilled water. This medium is actually limiting in thiamine and other growth requirements, but it is ideal for the scoring of mycelial interactions. A supplement of 1-5 gm per liter of yeast extract has frequently been added for spore isolation and the culture of biochemically deficient strains.

The minimal medium consisted of 20 gm dextrose, 1.5 gm asparagine, 120 μgm thiamine, mineral salts as in the complete medium, and 20 gm Noble's Special Agar per liter of distilled water. This medium has unexpectedly proved to be one of the best fruiting media of many tested.

Matings, either for fruit production or for the determination of mating type, have been made by placing small agar blocks containing mycelia of the appropriate strains about 2–3 mm apart on agar plates. Two matings were made per plate for determination of mating type; one mating per plate was made for fruiting.

Fruiting in compatible crosses may occur as early as four days after mating, or it may be delayed as long as two or three months, depending upon the fruiting competence of one or both of the mates. In a very few crosses, fruiting has not been achieved.

In the isolation of random samples of progeny from each cross, a sporulating fruit body was inverted over an agar plate, and the plate was checked microscopically for spores at intervals of a few minutes. After the appropriate period of collection, the spores were uniformly streaked over the agar surface with a bent glass rod. Eighteen to 24 hours later the spores had germinated, and well isolated germlings could easily be picked up under a dissecting microscope with a small, chisel-pointed needle. The isolated germlings (germinating spores) were transferred either four per agar plate or individually to agar slants in flat half ounce bottles. Germination counts were made in most cases at the time of isolation.

Determinations of mating type were made with various combinations of tester strains. The classical method uses as testers the four mating types possible in a particular cross, as follows:

	progeny			
testers	A^1B^1	A^1B^2	A^2B^1	A^2B^2
A^1B^1	−	F	B	+
A^1B^2	F	−	+	B
A^2B^1	B	+	−	F
A^2B^2	+	B	F	−

, where "+" represents the dikaryon; "F", the common *A* or "flat"; heterokaryon, "B", the "barrage" reaction; and "−", no discernible reaction (Papazian 1950). This method has the disadvantage of requiring four separate matings for the determination of the mating type

of each sibling of the sample. It accordingly has been but little used in the present studies.

A reliable test for mating type can be achieved with various combinations of two tester strains, including any two of the expected four types in the sample. One such method, which utilizes two tester strains each carrying a factor external to the cross to be tested (PAPAZIAN 1950), has been used in a majority of cases:

	progeny			
testers	A^1B^1	A^1B^2	A^2B^1	A^2B^2
A^1B^2	F	F	+	+
A^2B^1	B	+	B	+

The use of this less laborious test for mating type makes it necessary to apply a correction for incompatibility factor recombination in certain types of analyses. This correction is explained and justified below.

Identification of biochemical deficiencies was made by inoculating strains incapable of growth on the minimal medium onto media supplemented with the following substances, first in the listed groupings and then with individual members of the indicated groups:

A. biotin, choline, inositol, nicotinic acid, para-amino-benzoic acid;
B. calcium pantothenate, pyridoxin, riboflavin, thiazole, succinic acid;
C. cysteine, arginine, histidine, lysine, methionine;
D. phenylalanine, proline, serine, threonine, tryptophane; and,
E. guanine, adenine, hypoxanthine, xanthine, and uracil.

Scoring the progeny of crosses for biochemical deficiencies was done by transferring four or five sib mycelia, isolated and grown on complete medium, per plate to the necessary series of specifically supplemented media. In general, normal growth of deficient strains has only been achieved with surprisingly high levels of supplementation (e.g., 10^{-4} M for uracil, nicotinic acid, and arginine); biotin was a conspicuous exception to this generalization. Of the biochemical deficiencies, only four, *uracilless*, *niacinless-2*, *argininеless-1*, and *thiamineless*, have absolute requirements for their specific supplements. All of the remainder are "leaky" to a greater or lesser extent and in time slowly achieve full growth on minimal medium; the time of scoring is therefore critical.

OBSERVATIONS

Segregation and Recombination

The segregation of both *A* and *B* incompatibility factors and of suspected mutant characters has been determined and in each case the χ^2 test has been applied to the observed results to test their fit to a segregation ratio of 1:1.

Data relating to the segregation of the incompatibility factors are presented in Table 1. The *A* incompatibility factor is known to be a complex of two or more individual loci (PAPAZIAN 1951; VAKILI 1953), and recombination between component elements of the factor occurs at room temperature in frequencies ranging

TABLE 1

Segregation of incompatibility factors

Parental factors	Numbers of crosses	Sample	Segregation	χ^2	P
$A^1 \times A^2$	33	2539*	A^1 -1243 A^2 -1296	1.1	.29
$A^{35} \times A^{36}$	1	1078†	A^{35} -525 A^{36} -553	.72	.40
$B^1 \times B^2$	33	2618	B^1 -1321 B^2 -1297	.22	.66

* Original sample of 2618 less 79 (3%) *A*-factor recombinants.
† Original sample of 1192 less 114 (9.6%) *A*-factor recombinants.

from zero to 15 percent depending upon the specific combination of paired *A* factors (RAPER unpublished). A majority of the crosses examined here involved the factors A^1 and A^2. These factors recombine at a frequency of three percent to give new factors, a^1, a^2, etc., (collectively termed a^x) that are compatible with both parental factors (PAPAZIAN 1950; VAKILI 1953; RAPER unpublished). Since, in the two-strain test for mating type routinely used, a^x factors in the combinations, a^xB^1 and a^xB^2, were classified as A^2B^1 and A^2B^2, respectively, it is necessary to correct both the total sample and the A^2 class by the exclusion of three percent of the total. This correction has been applied to the data in line 1 of Table 1.

Segregation data are also given in Table 1 for a single large sample of progeny from a cross involving the factors A^{35} and A^{36}. This sample of 1192 was screened specifically for recombinant *A* factors, and these numbered 114 or 9.6 percent. In this case the necessary correction is applied only to the initial sample.

Although the *A* factor is known to involve a complex of loci, it serves as a perfectly regular genetic marker when correction is made for recombination between a specific pair of *A* factors. New *B* factors, presumed to be recombinant factors by analogy, have to date been found only in low frequency. In pairing between B^1 and B^2, new *B* factors are present among the progeny in a frequency of less than 0.5 percent and the appropriate correction does not significantly affect the results.

Table 2 comprises the data on segregation of the suspected mutant characters. In a few cases here, the results of a number of crosses have been excluded from the totals shown. Viability of the progeny has been found to be seriously impaired in certain crosses. This impairment may operate in the germination of the spores, in subsequent growth of the isolated germlings, or in both, and in the worst cases the final recovery has been as low as 40–50 percent. Such decreased viability is particularly noticeable in crosses involving two or more biochemical deficiencies and in crosses in which one parent carried any one of a number of specific morphological aberrations, such as puff and a majority of the UV morphs. In Table 2 in cases where data are available for two or more crosses, the results of those samples of progeny having low viability are excluded in which $P < .01$ in homogeneity tests with the remaining data; each item from which data have been excluded is marked with an asterisk (*). Only in one case, uracilless, are the results of more than a single cross omitted. In a few cases, adenineless, hypoxan-

thineless, and several UV morphs, data are available only from single crosses having low viability; the results from these are accordingly of questionable significance and have not been included here.

Two entries in Table 2 are devoted to segregation data for puff. These relate to the segregation of the character scored in the first entry as isolated mycelial progeny and in the second as germlings. The puff phenotype is the only aberrant character encountered that could be distinguished from wild type in the germling stage (Figure 3). Germination in all puff crosses was poor, counts revealing in most cases approximately equal numbers in each of three categories: (1) wild

TABLE 2

Segregation of mutant characters

Characters	Number of crosses	Total sample	Wild	Mutant	χ^2	P
Uracilless	19*	1558	825	733	5.4	.03
Niacinless-1	2	167	83	84	.006	.95
Niacinless-2	3†	272	144	128	.94	.36
Argininеless-1	7	683	340	343	.012	.90
Riboflavinless	2	113	56	57	.008	.95
Cholineless	3†	178	89	89	0.0	1.0
Biotinless	1	48	30	18	3.0	.08
Phenylalanineless	1	50	24	26	.08	.78
Guanine-Hypoxanthineless	1†	50	25	25	0.0	1.0
Unidentified deficiency	3	508	268	240	1.54	.21
Thiamineless	1	93	50	43	.54	.48
Streak	2	153	80	73	.32	.60
Fir	3†	368	190	178	.38	.59
Dwarf	1	50	35	15	8.0	<.001
	(3:1 seg.)				.76	.40
Fluff	2	140	70	70	0.0	1.0
Puff-1,2 (Recovered)	15‡	1338	727	611	10.2	<.001
(Germlings)	10‡	1990	959	1031	2.6	.13
Thin	1	159	89	70	2.26	.13
Feather	7	825	443	382	4.4	.04
Bubble	6	656	331	325	.05	.82
Early-blue	5	305	162	143	1.2	.30
Late-blue-1 + 2	1	82	33	49	3.12	.08
Late-blue-1	2	321	224	97	48.3	<.0001
Late-blue-2	2	366	224	142	18.0	<.0001
Pigmy	2	321	164	157	.142	.70
Concentric	3	521	260	261	.002	.95+
Felty	4	671	360	311	3.6	.06
Plumy	1	181	93	88	.13	.70
Restricted	1	159	70	89	2.3	.13
UV Morph.-1	1	60	28	32	.26	.63
UV Morph.-2	1	59	29	30	.016	.90
UV Morph.-3	1	114	63	51	1.26	.28

* Three crosses excluded because of low viability.
† One cross excluded because of low viability.
‡ Poor germination (ca. 65%) in both, low recovery in former.

type, (2) puff, and (3) ungerminated. At the germling stage, however, the essential equality of normal and puff segregants shows no bias in respect to the mutant character, whereas in the isolated mycelial progeny the puff class is significantly low. An additional loss of viability correlated with the character is thus indicated at a stage of development subsequent to germination. Two distinct puff loci are indicated by the recombination data (see below); this distinction is not revealed in the segregation data, and the crosses for both are summed in Table 2.

Only two aberrant characters, dwarf and late-blue, have been found to depart significantly from a segregation ratio of 1:1 in crosses of normal viability (> 90 percent). The dwarf phenotype may result from a pair of unlinked loci, since the observed segregation fits a 3:1 ratio with a P value of 0.40. The case of late-blue appears somewhat more complex.

The blue phenotype is characterized by the deposition of crystalline indigo in the medium (MILES, LUND, and RAPER 1956). The time of the appearance of the blue pigment, however, clearly differentiates early-blue and late-blue. In the former, indigo crystals are evident during early vegetative growth, while in the latter, the pigment only appears in aging cultures following the cessation of active growth. Late-blue consistently occurred in all but one cross in frequencies lying between 30 percent and 40 percent. In the exceptional cross, between wild type and a late-blue offspring of the Overholts-I dikaryon, late-blue segregants constituted somewhat more than half of the progeny, though the disparity was not significant. The data for this cross, however, are not compatible (homogeneity: $P < .0001$) with those from subsequent crosses involving late blue, in each of which one mate was a descendant of the original late-blue isolate. The suspicion that two loci responsible for the pigmentation were carried by the original isolate and that its descendants carried the factors singly appears to be confirmed by the pattern of recombination of the late-blue phenotype. Otherwise, the significantly low values for the late-blue segregants may indicate modifying factors that influence the deposition of indigo; the low values more likely reflect inaccuracies in scoring. The scoring of late-blue is difficult at best and frequently depends upon microscopic observation of crystalline indigo. Intensity of pigmentation is extremely variable and may often lie below the threshold for actual crystallization.

One further observation relating to the phenotypic expression and segregation of certain of the morphological mutants deserves brief mention. Three of these mutants, puff, fir, and feather, are extremely variable in their expression. In each case, however, the variation is not continuous but is represented by a limited number of variants, each of which originates as a distinct sector from the original mutant or from an already established sector. Rare progressive sectoring observed only in feather can restore the mutant to morphological and functional normality, yet from such restored mutants the original mutant form is regularly recovered in subsequent segregation. It appears most likely that these discrete variants represent the modification of mutant characters by suppressor mutations, but insufficient work has been done to establish the true basis of the phenomenon.

One of the primary objectives of the genetic work with *S. commune* has been to find genetic markers closely linked to the incompatibility factors. These were particularly desired to serve as analytical tools in the study of the genetic structure of the incompatibility factors themselves. The analyses for recombination that have been done have accordingly emphasized the relationships between the incompatibility factors and the various mutant loci, and these tests comprise the majority of the recombination data accumulated to date. Of the possible two-locus combinations between the characters studied, about one quarter have been tested. Summaries of these tests are presented in Tables 3 and 4.

TABLE 3

Assortment and recombination between incompatibility factors

Matings	Progeny					χ^2	P
	Total number	A^1B^1	A^1B^2	A^2B^1	A^2B^2		
$A^1B^1 \times A^2B^2$	1458	367	345	340	406	7.43	.06
$A^1B^2 \times A^2B^1$	1082	282	249	293	258	4.49	.22
Summed	2540	649	594	672	664	4.24	.25
$A^1B^1 \times A^2B^2$		Parental		Nonparental			
and	2540	1315		1225		3.2	.08
$A^1B^2 \times A^2B^1$							

The tests for independent assortment and recombination between the incompatibility factors are given in Table 3. These data are taken only from crosses $A^1B^1 \times A^2B^2$ and $A^1B^2 \times A^2B^1$, and a correction is applied for recombinant *A* factors as described above. The results of these tests indicate no linkage between the two members of the bifactorial incompatibility system and are in complete agreement with the findings of earlier workers for this and other species of tetrapolar Hymenomycetes (KNIEP 1923; BRUNSWIK 1924; OIKAWA 1939).

Data relating to all other tests for recombination—between the incompatibility factors and other loci and among the extraincompatibility loci—are given in Table 4.

The recombination data indicate linkage of the *A* factor with six characters, streak, late-blue-1, pigmy, restricted, unidentified deficiency, and concentric, and of the *B* factor with one, fir. In none of the cases, however, is the linkage close, and the utility of these markers in the resolution of the structure of the incompatibility factors is doubtful, although two, restricted and pigmy, will be tested in the near future with a pair of *A* factors that yield recombinants in high frequency.

In addition to indicating instances of linkage among other characters, the analyses for recombination revealed an unsuspected heterogeneity in two of the mutant characters. In each of two cases the data clearly indicate two unlinked mutant loci as responsible for a single phenotypic expression. The first of these, puff, is an exceptionally distinct morphological abnormality, yet in the combina-

TABLE 4a

Assortment and recombination between incompatibility factors and mutant characters and between mutant characters

Characters	Ratio: parental/total sample from mating to		
	A-factor	*B*-factor	Uracilless
Uracilless	628/1190	595/1190	....
Niacinless-1	92/167	88/167	32/59
Niacinless-2	127/258	118/229	238/332†
Argininеless-1 (1)	281/557	255/547	212/392
Riboflavinless	57/113	55/109	31/49
Cholineless (2)	56/117	59/117	54/103
Biotinless	22/48	21/48	30/48
Phenylalanineless	21/50	21/50	21/50
Guanineless	23/50	22/50	38/50†
Unidentified deficiency	250/521	287/520	....
Thiamineless	50/93	....	....
Streak	78/92†	48/92	30/61
Fir	216/455	306/455†	67/139
Fluff	75/136	68/136	....
Puff-1 (3)	351/729	375/729	304/605
Puff-2 (4)			109/147†
Thin	32/62	33/62	46/62
Feather (5)	204/403	221/403	35/62
Bubble (6)	121/256	133/256	77/147
Early blue	82/152	75/152	56/77†
Late blue-1 (7)	285/403†	206/403	....
Late blue-2 (8)	199/366	184/366	....
Pigmy (9)	264/321†	169/321	....
Concentric (10)	241/519	279/519	....
Felty (11)	190/360	260/498	....
Plumy	86/181	90/181	....
Restricted (12)	102/159*	82/159	....
UV morph.-1	31/60	36/60	29/60
UV morph.-2	....	....	39/56

tions with uracilless and niacinless-2 the mutant puff in one particular cross recombined in a very significantly different manner from the other crosses involving puff (homogeneity: $P < .0001$). Two puff mutants of different origins were used here: puff-1 came originally from a common *A* heterokaryon of the $A^1A^2B^1B^2$ (Argonne, Ill.) stock; puff-2 came from a common *A* heterokaryon of the $A^9A^{10}B^9B^{10}$ (Big Sur, Calif.) stock. The recombination data (Table 4) show puff-2 to be linked to uracilless and niacinless-2 while puff-1 is not linked to either of these loci. Two unlinked loci thus appear to be responsible for the puff phenotype.

The second case, late blue, equally clearly differentiates another phenotypic grouping into two differently recombining classes. In this case the mutant character can in all cases be traced back to a common source, a single monosporous

offspring of the $A^{15}A^{16}B^{15}B^{16}$ (Overholts-I) stock. That two alternative loci may be jointly or singly responsible for the delayed deposition of indigo is indicated by both segregation data (Table 2) and recombination data (Table 4). In the recombination tests, the distinction between late-blue-1 and late-blue-2 depends upon different recombination frequencies with five loci, the *A* factor, pigmy, unidentified deficiency, felty, and plumy. Certain peculiarities in the pattern of recombination between different *A* factors could account for the distinction in respect to linkage with the *A* factor—for the different clasess of late blues coincide with different pairings of *A* factors—but recombination with the extraincompatibility loci, pigmy and unidentified deficiency, should then be unaffected.

The relationship between argininеless-1 and argininеless-2 has been of some interest since neither could be restored by proline, ornithine, or citrulline, but the growth of their dikaryon on minimal medium showed them to be nonallelic. Recovery of the progeny of the single argininеless-1 × argininеless-2 cross that we have been able to fruit was very low. Wild type recombinants were present in sufficient frequency to indicate no close linkage, but the data were inadequate to distinguish between distant linkage and independent assortment between the two loci.

Somewhat more than half the loci tested show linkage with one or more other loci. These may be grouped into five loose linkage groups within which the approximate order is as follows:

1. restricted, *A* factor, pigmy, streak, late blue-1, unidentified deficiency, concentric;
2. *B* factor, fir;
3. uracilless, niacinless-2, guanineless-hypoxanthineless, puff-2, early-blue;
4. niacinless-1, argininеless-1, cholineless; and
5. felty, late-blue-2, plumy.

TABLE 4b

	Matings	Ratio: parental/total sample		Matings	Ratio: parental/total sample
(1)	Arg-1 × Nic-1	107/108‡	(6)	Bubble × Nic-2	63/147
	Arg-1 × Nic-2	58/129	(7)	L. blue-1 × Unident.	124/162‡
	Arg-1 × Chol.	80/114‡		L. blue-1 × Pigmy	242/344‡
	Arg-1 × Puff-1,2	123/245		L. blue-1 × Felty	80/162
	Arg-1 × Thin	34/62	(8)	L. blue-2 × Unident.	95/185
	Arg-1 × Feather	29/62		L. blue-2 × Felty	163/185‡
(2)	Chol. × Ribo.	27/64		L. blue-2 × Plumy	173/181‡
(3)	Puff-1 × Nic-2	56/129	(9)	Pigmy × Unident.	85/162
	Puff-1 × Streak	30/61	(10)	Concent. × Unident.	455/519‡
	Puff-1 × E. blue	173/324		Concent. × Pigmy	82/162
(4)	Puff-2 × Nic-2	101/147‡		Concent. × Felty	177/334
	Puff-2 × Bubble	85/147		Concent. × L. blue-1,2	168/347
	Puff-1,2 × Nic-1	55/103	(11)	Felty × Unident.	181/347
(5)	Feather × Thin	28/62	(12)	Restrict. × Pigmy	96/159*

* $P<0.01$.
‡ $P<0.001$.

These data are drawn from a large number of crosses involving strains of widely assorted parentage, and it is not surprising that the data are insufficient to permit the construction of accurate maps of any of the groups. There are, however, no qualitative inconsistencies in the recombination data in respect to the assignment of the various loci to the several linkage groups.

DISCUSSION

The study reported here represents an extensive survey that has extended over a period of years and includes the observations of a number of workers. The results are in many ways fragmentary; e.g. they represent only about a quarter of the possible two-locus comparisons for recombination. Three facts account for this seeming deficiency: (a) the limitations of time and effort available to devote to a secondary facet of the overall work on sexuality in Schizophyllum, (b) the "impossibility" of so many crosses of particular interest due to the unilateral mating behavior of most of the morphological mutants, and (c) the exclusion of data from many crosses in which low viability has made the results of analyses questionable or meaningless.

The evidence from the recombination data of five linkage groups gives no indication of the number of chromosomes in view of the large number of unlinked loci to which no group assignment can be made. The fact that 13 of the loci examined here can be so widely scattered as to show no linkage whatever, strongly suggests that the five linked groups represent a relatively small portion of the entire chromosomal complement. The number of these groups cannot, therefore, be construed as a contradiction of the haploid complement of three chromosomes described by EHRLICH and MCDONAUGH (1949) in the only published study of meiosis in *S. commune*. Any, or for that matter all, of the linkage groups established here could conceivably occupy distant segments of the same chromosome.

The incompatibility factors were originally interpreted as multiple-allelomorphic series in which frequent "mutations" gave rise to new factors (KNIEP 1922, 1923); but even interpreted in this way the number of alternate states at each locus made them rather exceptional (WHITEHOUSE 1949). Recent work (PAPAZIAN 1951; VAKILI 1953; RAPER 1953) has demonstrated the multilocus structure of the *A* factor, and current work in this laboratory reveals the *A* factor to be an unusually complicated genetic system. A preliminary analysis of collections from all over the world indicates about 340 alternate *A* factors in nature, while specific *B* factors are far less numerous, probably of the order of 60. In many ways the *A* factor behaves as a "pseudoallelic" system, yet the frequency of intrafactor recombination may be as high as 15 percent. The ultimate objective in this work, a demonstration of the mode of action of the system in determining so many alternate factors of such rigid specificity, is a distant one, but its possible attainment is worth considerable effort.

Perhaps of comparable genetic interest and significance are the opportunities for the study of internuclear reactions afforded by the variety of incompatibility-determined heterokaryons that occur only in tetrapolar species. The pattern of

nuclear migration, the rate of nuclear division (SNIDER and RAPER 1958), the final nuclear ratio (SNIDER and RAPER unpublished), genetic complementation between associated genomes, metabolic competence, and morphological habit (RAPER and SAN ANTONIO 1954), vary widely in heterokaryons of the different types determined by the four basic combinations of incompatibility factors, i.e., homo- and heterozygotes at either or both of two "loci." The continuing study and comparison of the different heterokaryons in respect to these several aspects of interaction hold particular promise in the area of physiological genetics.

These problems can of course be studied only in tetrapolar species, and of these, Schizophyllum is one of the best known and most intensely studied.

SUMMARY

Two major conclusions may safely be drawn from the present study with Schizophyllum in spite of the limitations given above.

1. From the standpoint of basic genetic behavior, there appears to be nothing exceptional about Schizophyllum. The incompatibility factors, *A* and *B*, concerning which an intensive study is in progress, segregate and recombine in a perfectly regular manner although their genetic structure is still unknown; their inclusion here serves primarily to test their relationship with other factors. Otherwise, of the morphological and biochemical aberrations for which reliable data are available—one or more crosses having recovery values of 90 percent or higher—only two, dwarf and late-blue, apparently fail to segregate as single loci, and the over-all pattern of recombination, although sketchy, is completely consistent. An extension of the study, filling in the existing gaps and testing a large number of additional characters that are available, could without doubt provide the precise information requisite to complete chromosome mapping.

2. From the standpoint of material for the study of certain basic genetic problems, Schizophyllum offers exceptional promise. To be sure, the intricacies of mating behavior imposes certain limitations on the use of Schizophyllum as a general genetic material. These intricacies, however, are the essential tools for the resolution of those particular phenomena that are peculiar to the organism and/or to the group to which it belongs. Two such phenomena are of considerable genetic interest and their significance transcends their novelty as unusual features confined to and characteristic of a restricted phylogenetic group.

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THE MEIOTIC BEHAVIOR OF GROSSLY DELETED X CHROMOSOMES IN DROSOPHILA MELANOGASTER[1]

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IT HAS been known for many years that the proximal heterochromatin of the X chromosome functions in disjunction. In the early paper of MULLER and PAINTER (1932), in which they demonstrate that the proximal half of the X is genetically inert, the only function that they can attribute to this region other than that directly assignable to the normal allele of *bb* is the control of disjunction of the X chromosome from the Y chromosome at spermatogenesis. It was the purpose of this investigation to examine in more detail the role of the proximal heterochromatin of the X chromosome in sex chromosome disjunction. The experiments to be reported were suggested by a paper by GERSHENSON (1940) and are in part a repetition, and in part an extension, of his work. GERSHENSON's approach was essentially to fractionate the proximal heterochromatin in a series of free X chromosome duplications, and to examine the influence of these duplications on the disjunction of normal homologs. Because his extensive investigations are published in Ukranian[5] and are thus largely unavailable to English-speaking geneticists, they will be reviewed briefly here.

It is possible, by irradiation of a normal X chromosome, to induce the loss (as an interstitial deletion) of a major portion of the euchromatin with or without sections of the adjoining proximal heterochromatin. The resulting deleted chromosome [Del(1)] consists of the centromere of the X chromosome and some or all of the proximal X heterochromatin plus a portion of the euchromatic tip marked with y^+. The deleted chromosome may also carry a portion of the proximal euchromatin; the amount of euchromatin carried by such a chromosome can be so small as to have a negligible effect on viability when hyperploid in either sex. Such chromosomes are carried as free duplications [Dp(1;f)] marked with y^+. GERSHENSON measured a series of these duplications cytologically and studied their effect on disjunction of X from X in females and of X from Y in males. He

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[4] Operated by Union Carbide Nuclear Company for the United States Atomic Energy Commission.

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reports that the duplications fell into three cytologically well-defined classes: about 0.5 times, 0.25 times, and 0.1 times the length of a normal X. He was able to rule out a significant contribution of the euchromatic portion of the duplications to the measurable differences in length, and consequently he ascribes the difference between the long and intermediate duplications to *Block A* (0.25 times the length of a normal X) and that between the intermediate and the short duplications to *Block B* (0.15 times the length of a normal X). On the basis of the disjunction in +/+/ Dp and +/dl–49/Dp females, the duplications could be divided into four classes characterized by relatively high, intermediate, low, and negligible frequencies of secondary nondisjunction. The correlation between these classes and cytological length was good, but not exact. GERSHENSON argued that the amount of secondary nondisjunction is a measure of the interference by the duplication with normal disjunction of the X chromosomes and consequently of the pairing affinity of the duplication for the X chromosome. Proceeding from this premise he concluded that the high nondisjunction duplications carried a pairing site (presumably equivalent to the collochores of COOPER 1941, 1951) that the intermediate, low, and negligible nondisjunction duplications lacked; similarly, that the intermediate nondisjunction duplications contained a pairing site that the low and negligible nondisjunction duplications lacked; and finally, that the low nondisjunction duplications contained a site that the negligible class lacked. It was also possible to infer three pairing sites on the basis of disjunction in X/Y/Dp males. The correlation among the four classes of duplications defined by nondisjunction in females and the four defined by nondisjunction in males was not perfect: it indicated, in fact, that there are two pairing sites in the proximal X heterochromatin that function both in males and in females, one that functions in females only, and one that functions in males only. The designation and location of these pairing sites are diagrammed in Figure 1.

GERSHENSON further took those duplications causing maximal nondisjunction as demonstrating 100 percent of the potential effect of the proximal heterochromatin; then by observing the effect of removing the pairing sites one at a time (proceeding from the most distal, proximally), he was able to estimate the rela-

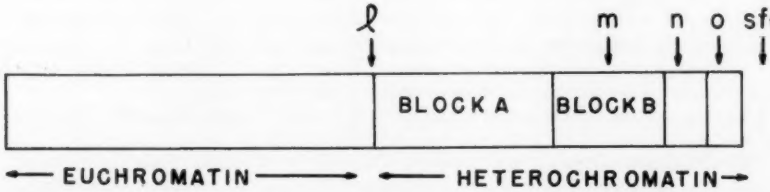

FIGURE 1.—A map of pairing sites in the proximal X chromosome heterochromatin as inferred by GERSHENSON (1940) from measurements of the rates of secondary nondisjunction induced in males and females by heterochromatic X chromosome deletions and by cytological measurements of these deletions in oogonia (figure modified from FIGURE 48 in GERSHENSON 1940).

tive effect, and inferentially the relative pairing affinity, of each pairing locus in both males and females. These estimates are tabulated in Table 1.

We undertook these experiments to check several points that are not clear from GERSHENSON's paper. If, as he postulates, a high rate of secondary nondisjunction effected by a duplication is indicative of a high pairing affinity between the duplication and its normal homologs, it can be further postulated that such a high pairing affinity would evoke a low incidence of primary nondisjunction, i.e., a reciprocal relation might exist between the incidence of primary and secondary nondisjunction. It is well established that reciprocal products of primary nondisjunction are not recovered with equal frequencies. This has been attributed by SANDLER and BRAVER (1954) to the meiotic loss of unpaired homologs. The possible role of meiotic loss of unpaired duplications in GERSHENSON's experiments is not easy to assess. Consequently, the effect of a newly derived series of duplications on secondary nondisjunction in +/dl–49/Dp females, and on primary nondisjunction in attached-X/Dp females and in $Y^sX \cdot Y^L$/Dp males was determined.

TABLE 1

The average relative synaptic activity of different groups of genes of the inert region of the X chromosome in females and males of Drosophila melanogaster

Group of genes	Synaptic activity in females (%)	Synaptic activity in males (%)
l	70.2	18.3
m	0.0	13.1
n	13.5	69.6
o	16.3	0.0

The synaptic activity of the entire inert region of the X chromosome is assumed to be 100 percent (Table 48 of GERSHENSON 1940).

In general, the duplications studied in +/dl–49/Dp females fell into groups in much the same way as did those studied by GERSHENSON. Also, the predicted reciprocal relation between the incidence of primary and secondary nondisjunction is fully realized. It was further determined that meiotic loss of the duplications does occur, but that in general it is not a major factor to be considered in the interpretation of the nondisjunction data. Finally, an entirely unexpected phenomenon was encountered in the results from some of the $Y^sX \cdot Y^L$/Dp males. In these cases the incidence of the $Y^sX \cdot Y^L$ chromosome (=XY) among the progeny of XY/Dp males is reduced to much below 50 percent (as low as 16 percent in one case). It can be shown that this reduced recovery is not the result of meiotic loss of the XY chromosome, nor can it be attributed to zygote mortality; it must be, therefore, that functional gametes bearing reciprocal products of meiosis do not function equally in fertilization (see also NOVITSKI and I. SANDLER 1957).

Origin and characterization of duplications

The thirteen duplications used in this study were derived by DR. A. WELTMAN in the following manner: Canton-S males were X-irradiated with approximately

4500r and crossed to $y\ v\ car$/Y females. Non-y daughters were collected and were presumed to contain a duplication of the type described in the introduction. Each was crossed to $Y^sX \cdot Y^L$, *In(1)EN*, $y\ B$/0 males. The y^+ *B* males recovered in the following generation were crossed, where possible, to exceptional (y^+) sisters and otherwise to regular (y) sisters. This mating scheme automatically eliminates any duplication that is lethal or sterile in XY/Dp males (this, incidentally, included all duplications carrying car^+). Furthermore, any duplication that contained the normal allele of *pn* (0.8) was arbitrarily considered to carry excessive distal euchromatin and was discarded. Each of the remaining duplications was tested for the presence of the normal alleles of *ac*, *sc*, and $su\text{-}w^a$ distally and of *su-f* and *bb* proximally. The results of these tests are summarized in Table 2.

TABLE 2

Genetic constitution of deletions

	Loci carried						
	Distally		Proximally		Translocation with		
Deletions	*sc*	$su\text{-}w^a$	*su-f*	*bb*	II	III	IV
Dp(1;f)3	+	—	+	+	—	—	—
Dp(1;f)10	+	?	—	—	—	—	—
Dp(1;f)12	+	+	+	+	—	—	—
Dp(1;f)18	+	+	—	+	—	—	—
T(1;?)42	+	?	—	+	*	*	*
T(1;3)51	+	+	—	+	—	+	—
Dp(1;f)52	+	—	+	+	—	—	—
Dp(1;f)122	+	—	—	+	—	—	—
T(1;3)142	+	+	+	+	—	+	—
Dp(1;f)164	—	—	—	—	—	—	—
Dp(1;f)167	+	+	+	+	—	—	—
T(1;4)174	+	—	—	—	—	?	+
Dp(1;f)179	+	+	—	—	—	—	—

For general discussion, see text.

* This deletion was lost before translocation tests could be performed. The fact that the primary exceptional females from XY/Del(1)142♂ × $y\ w$/0 ♀ had outstretched wings (probably viable hyperploids) suggests that this duplication is also associated with an X autosome translocation.

Each duplication used in these studies was also tested for linkage between y^+ and the autosomes. Two of the duplications showed linkage with chromosome 3 and a third was linked with chromosome 4. In the two T(1;3)'s, bb^+, as well as y^+, was linked to chromosome 3. These then are peculiar translocations in that they carry the tip and the centric region of the X chromosome, but not the intervening euchromatic region from pn^+ through car^+. They may be described as "deletional translocations." The X chromosome was apparently broken distally and proximally and the third chromosome broken once; the tip of the X was then attached to the centric portion of chromosome 3 and the acentric portion of the third was attached to the centromere of the X. The interstitial portion of the X was lost as an acentric. That these translocations are of the cytological constitu-

tion expected from the above events has been confirmed in polytene preparations. Is it possible that the T(1;4) is also a deletional translocation in which X centromere portion either is not marked with bb^+ or has been lost. It might be noted that there are no bona fide cases of what ABRAHAMSON *et al.* (1956) have termed "half-translocations" among the aberrations reported here; this is, of course, to be expected, since meiosis has not intervened between irradiation and sampling. These aberrations will henceforth be referred to generically as deletions [Del(1)] of which there are two structural types—deletional translocations (e.g., T(1;3) 142) and free duplications (e.g., Dp(1;f)118).

RESULTS

Disjunction in females

The data from crosses of females of constitution *y v f*/Del and *y v/In(1)*dl-49, *y* fa^s/Del with Y^sX:Y^L, *In (1)EN*, *y B*/0 males are presented in Tables 3 and 4. A comparison of the frequencies of nondisjunction in the two situations reveals a remarkably good inverse correlation. The deletions arranged in order of decreasing frequency of secondary nondisjunction in X/*In(1)*dl-49/Del females (see Table 5) correspond reasonably well with an arrangement in order of increasing primary nondisjunction in attached-X females; furthermore, if the known and suspected translocations are eliminated from this list, the inverse relation becomes nearly exact. It can be seen from Table 5 that the frequencies of nondisjunction do not form a continuous distribution; however, such a small sample of duplications cannot be considered to demonstrate a discontinuous dis-

TABLE 3

Progeny of y v f/*Del* ♀ × Y^SX-Y^L, y B/*0* ♂

Deletion	Constitution of egg nucleus and phenotype of progeny				Primary nondisjunction
	XX	Del	XX/Del	0	[XX/Del + 0 / total]
	y v f ♀	*B* ♂	*v f* ♀	*y B* ♂	
0	830	..	..	795	...
Dp(1;f)3	1137	1004	43	22	0.03
Dp(1;f)10	866	324	246	726	0.45
Dp(1;f)12	1447	1214	78	35	0.04
Dp(1;f)18	1391	1000	46	64	0.04
T(1;?)42	221	181	16	5	0.05
T(1;3)51	599	583	234	192	0.26
Dp(1;f)52	863	709	38	18	0.03
Dp(1;f)122	1390	1262	93	78	0.06
T(1;3)142	666	592	310	350	0.34
Dp(1;f)164	706	491	349	320	0.36
Dp(1;f)167	1265	1061	41	39	0.03
T(1;4)174	275	213	266	211	0.49
Dp(1;f)179	1070	730	213	228	0.20

TABLE 4

Progeny of y v/In(1)*dl-49*, y fa^n/*Del* ♀ × *Y^{S}X-Y^L*, y B/*0* ♂

Deletion	Constitution of egg nucleus and phenotype of progeny: X, *y B* ♀	X, *y* ♂	X/Del, *B* ♀	X/Del, + ♂	X/X, *y* ♀	Del, *B* ♂	Secondary nondisjunction $\left[\frac{2(X/X+Del)}{total+X/X+Del}\right]$
Dp(1;f)3	1261	1270	1136	1344	287	255	0.178
Dp(1;f)10	1900	2058	522	622	4	4	0.003
Dp(1;f)12	848	921	795	925	213	202	0.192
Dp(1;f)18	1243	1479	1233	1398	237	185	0.136
T(1;?)42	629	767	541	672	1109	974	0.615
T(1;3)51	1385	1692	1351	1615	58	57	0.037
Dp(1;f)52	1072	1288	1115	1198	191	162	0.131
Dp(1;f)122	1464	1831	1395	1710	189	140	0.093
T(1;3)142	1643	1913	1477	1661	43	41	0.024
Dp(1;f)164	1737	1983	1482	1815	3	4	0.002
Dp(1;f)167	701	771	717	576	165	109	0.165
T(1;4)174	744	697	746	673	1	1	0.001
Dp(1;f)179	957	1140	911	976	19	14	0.016

TABLE 5

Summary of disjunctional behavior of deletions separated according to structure and arranged in order of decreasing secondary nondisjunction in females

Deletion	Behavior in females: Primary nondisjunction	Secondary nondisjunction	Secondary nondisjunction (GERSHENSON, 1940)*	X/Del: X/Del + X	Proximal constitution: *su-f*+	*bb*+	Behavior in males: Primary nondisjunction	XY + XY/Del: Total
Dp(1;f)12	0.04	0.193	....	0.493	+	+	<0.001	0.474
Dp(1;f)3	0.03	0.178	....	0.495	+	+	0.001	0.445
Dp(1;f)167	0.03	0.163	....	0.506	+	+	<0.001	0.279
Dp(1;f)52	0.03	0.131	....	0.495	+	+	<0.001	0.474
Dp(1;f)18	0.04	0.136	....	0.492	—	+	<0.001	0.453
Dp(1;f)122	0.06	0.093	0.086(6) 0.032(5)	0.485	—	+	<0.001	0.476
Dp(1;f)179	0.20	0.016	0.017(6)	0.474	—	—	0.001	0.246
Dp(1;f)164	0.36	0.002	0.001(2)	0.470	—	—	0.485	0.460
Dp(1;f)10	0.45	0.003	....	0.224	—	—	0.613	0.361
T(1;?)42	0.05	0.616	....	0.465	—	+	0.037	0.336
T(1;3)51	0.26	0.037	....	0.491	—	+	0.002	0.335
T(1;3)142	0.34	0.024	....	0.469	+	+	0.040	0.159
T(1;4)174	0.49	0.001	....	0.496	—	—	0.482	0.423

* Frequencies of secondary nondisjunction in females characteristic of GERSHENSON's four classes of duplications. Each frequency is the average of the number of duplications indicated in parentheses.

tribution. The classes of deletions indicated by the incidence of secondary nondisjunction in GERSHENSON's crosses ($y\ sc/In(1)$dl-49, $y\ w\ lz$/Del $\times\ y\ v\ f\ B$/Y) correspond quite well with those indicated by the present experiments except that his observed frequencies of nondisjunction are generally lower than those being reported here (see also Table 5). This might be simply because the rates of nondisjunction are inherently different owing to differences in genetic background, or it might be that the deletions showing the highest rate of secondary nondisjunction in these experiments have no counterpart in GERSHENSON's experiments; i.e., they may possess an additional pairing site. The latter possibility is not unreasonable because these deletions would be retained only rarely by the methods available to GERSHENSON, which relied on the recovery of a primary exception (attached-X/Y/Del), from the attached-X female in which the deletion was originally recovered, for establishment of a line. Table 3 demonstrates that such reliance would definitely result in the recovery of a biased sample of deletions characterized by higher average primary nondisjunction from an attached-X chromosome. In these experiments the XY chromosome obviates this difficulty.

On the basis of their disjunction from the attached-X chromosome (Tables 3 and 5), the duplications (exclusive of the translocations) do not form a continuous distribution, and the discontinuities in this classification coincide with those based on the secondary nondisjunction data.

An examination of the constitution of the duplications showing these different behaviors reveals no correlation between euchromatic content (*sc*, *su-w*^{a}, *su-f*) and disjunctional behavior. All those duplications characterized by low nondisjunction from the attached-X and high secondary nondisjunction of +/dl-49 carry the *bb*$^+$ region of the X chromosome. Duplications associated with high nondisjunction from the attached-X and normal disjunction of + from dl-49, on the other hand, do not carry *bb*$^+$. These observations are similar to those of GERSHENSON.

As stated in the introductory portion of this report, one of the purposes of these studies was the examination of the behavior of the deletions with respect to the phenomenon of meiotic loss. One simple index of meiotic loss of a deletion is the depression, below 50 percent, of its recovery. It will be realized that such a depression may also have its origin in a decrease in viability, owing to hyperploidy. Although the deletions studied here were selected for minimal euchromatic content, there is no possibility of a clear distinction between meiotic loss and inviability as the cause of depressed recovery of deletion bearing individuals, but in these cases arguments can be brought to bear that render unlikely a significant influence of inviability. In the first place, the effect of duplication on the viability of a hyperploid individual should be correlated with the genetic content of the duplication such that inviability is associated with certain factors carried in the duplication. In these experiments there is no obvious relation between the recovery of a particular deletion and its genetic (i.e., euchromatic) content. Secondly, gen-

eral experience suggests that the effect of X chromosome hyperploidy on viability would be greater in males than in females. In only one of the cases examined among the present series of deletions [Dp(1;f)167], is there any evidence of a greater discrepancy in recovery of the deletion in males than in females. In this case the ratio of X to X/Del ova recovered from +/dl-49/Del females is 701:717 among females and 771:576 among males. It is probably a reasonable approximation to say that, except for Dp(1;f)167, the discrepancies observed in the deletion-bearing classes are not attributable to inviability.

Among the exceptional progeny from crosses of attached-X/Del females, any discrepancy in recovery of the deletion owing to events transpiring in the female is obscured by the unequal production of XY and 0 sperm caused by meiotic loss of the XY chromosome in the male. However, +/dl-49/Del females produce X and X/Del ova as reciprocal products of regular disjunction, and by analogy with the known behavior of the Y chromosome during regular disjunction in X/X/Y females (SANDLER and BRAVER 1954), meiotic loss of the deletion should occur. The ratio in which X and X/Del ova are recovered is unaffected by whether they are fertilized by an XY- or a 0-bearing sperm. Consequently, the recovery of the deletion among the regular progeny of X/X/Del females was selected as a measure of loss of the deletion. Except for Dp(1;f)167, where only regular females are used because of the suspected inviability of X/Del males, both sexes are lumped for these comparisons. It is found that there is a correspondence between recovery of the deletion and its disjunctional behavior. Deletions characterized by low primary nondisjunction and high secondary nondisjunction are recovered in close to 50 percent of the gametes, whereas those characterized by high primary and low secondary nondisjunction are recovered in less than 50 percent of the gametes.

It is of interest to compare the effects of X heterochromatin with the effect of the Y chromosome on disjunction in females. The normal segregation of a Y from an attached X is a common observation and is comparable to the segregation of Dps(1;f)3, 12, 18, 52, 122, and 167 from attached X chromosomes. The effect of a Y chromosome on the disjunction of *In(1)*dl-49 from a normal X has been calculated from the data of STURTEVANT and BEADLE (1936) in the same manner as was done in Table 4. The frequency of secondary nondisjunction observed by STURTEVANT and BEADLE was 0.61. This value is much greater than that for any of the deletions studied either in the present paper or by GERSHENSON with the exception of Del(1)42 (0.62), which is thought to be associated with a translocation. This observation agrees very well with the predictions from COOPER's theory (1948) of secondary nondisjunction in female *Drosophila melanogaster*, which states that nondisjunction of two X's from a Y is the result of non random segregation from an X-Y-X trivalent in which each X pairs with a different arm of the Y. Such a trivalent cannot be formed when the extra element is one armed; consequently, a bivalent (one X plus the extra element) and univalent (the other X) are formed, with the chromosomes of the bivalent separating normally and the univalent proceeding at random, as originally postulated by BRIDGES (1916) to

explain secondary nondisjunction. Both models utilize an X/X bivalent with the extra element univalent to explain regular disjunction. BRIDGES' model predicts a maximum rate of secondary nondisjunction of 50 percent, which is not exceeded by any of the duplications studied, but which is exceeded when T(1;?)42 or the Y is the extra element. The reason for the high frequency of secondary nondisjunction observed in the presence of T(1;?)42 is difficult to explain. To be consistent with COOPER's theory, the element that is homologous to the X's should be two armed. Since T(1;?)42 carries a normal allele of *bb*, we concluded that it carried the centric region of the X chromosome; the possibility exists, however, that T(1;?)42 is a T(X;Y) and that the bb^+ allele was derived from the Y. Unfortunately, this line have been lost and this possibility cannot be checked. However, it is difficult to understand how segregants from such a translocation could yield the aneuploid-looking exceptions from XY/Del(1)42 males by which this deletion was originally judged to be a translocation.

Disjunction in males

Primary nondisjunction of the deletions from an XY chromosome was measured in the following mating: $Y^SX{\cdot}Y^L$, *In(1)EN*, *y B*/Del ♂ × *y w*/0 ♀; the data are presented in Table 6. When the relative frequencies of primary nondisjunction of the deletions are examined in Table 5, it is evident that a correlation exists between the behavior of the different deletions in males and in females. The single discontinuity in the distribution of frequencies of nondisjunction divides the duplications into a group that separates almost perfectly from the XY chromosome and a group that appears to separate randomly from it.

TABLE 6

Progeny of y w/0 ♀ × $Y^SX{\cdot}Y^L$, y B/*Del* ♂

	Constitution of sperm nucleus and phenotype of progeny				Primary nondisjunction	Recovery of XY
	XY	Del	XY/Del	0	$\left[\frac{\text{XY/Del}+0}{\text{Total}}\right]$	$\left[\frac{\text{XY}+\text{XY/Del}}{\text{Total}}\right]$
Deletion	*y B* ♂	*w* ♀	*B* ♂	*y w* ♀		
0	816	...	..	945	....	0.463
Dp(1;f)3	2296	2854	0	7	0.001	0.446
Dp(1;f)10	1179	467	357	2246	0.613	0.361
Dp(1;f)12	2502	2769	0	2	<0.001	0.475
Dp(1;f)18	2663	3208	0	2	<0.001	0.453
T(1;?)42	564	1055	0	62	0.037	0.336
T(1;3)51	682	1352	1	3	0.002	0.335
Dp(1;f)52	2653	2940	0	2	<0.001	0.474
Dp(1;f)122	2103	2318	0	1	<0.001	0.476
T(1;3)142	197	1016	4	47	0.040	0.159
Dp(1;f)164	1231	1513	1219	1368	0.485	0.460
Dp(1;f)167	1299	3349	0	3	<0.001	0.279
T(1;4)174	967	1302	886	1226	0.482	0.423
Dp(1;f)179	592	1814	0	3	0.001	0.246

With one exception, there is no evidence of loss of the deletions in XY/Del males; in fact, there are generally more deletion-bearing progeny recovered than not. In Dp(1;f)10, however, only 19.4 percent of the progeny carry the duplication; this compares well with 22.4 percent, which is the recovery of Dp(1;f)10 among the progeny of X/X/Dp females. The striking feature about the data from XY/Del males, however, is that in every line the XY chromosome is recovered in fewer than half of the progeny. In some cases the deficiency of XY bearing classes is severe, and it is quite likely that this apparent loss of the XY obscures any loss of the deletions that might be occurring. Table 4 shows that whereas there has been a correlation between the behaviors measured with respect to the different lines up to this point, there is no logical seriation of the apparent loss of XY. Deletions with high and low synaptic activity show both high and low recovery of XY. These departures from expected ratios were originally considered to be the result of meiotic loss of the XY chromosome. The observations were not, however, in accord with the ideas of SANDLER and BRAVER on meiotic loss of $In(1)sc^{4L}$, sc^{8R} in $In(1)sc^{4L}$, sc^{8R}/sc^{8}·Y males in three respects. In the first place, loss of the XY was not correlated with the disjunction of this chromosome from its homolog; situations with nearly perfect disjunction (Dp(1;f)179) or nearly random disjunction (Dp(1;f)10) showed a grossly deficient recovery of XY. In the second place, high loss of the XY chromosome was not accompanied by the recovery of gametes from which it had been lost; that is to say, the virtual absence of a nullo XY, nullo Dp class from Dp(1;f)167 is incompatible with the loss of XY during meiosis as an explanation of the deficiency of this chromosome among the progeny. Finally, it is impossible to account for recovery of the deletion in more than half of the progeny (as high as 80 percent in one case) by invoking the phenomenon of meiotic chromosome loss. We concluded, therefore, that the discrepancy must be the result of gametic loss, either before or after fertilization. The latter should be detectable by the failure of an appreciable proportion of the zygotes formed to develop into adults. Consequently, the proportion of eggs fertilized by XY/Del males (from lines characterized by low XY recovery) that developed into adult flies was determined. If the observed discrepancy were the consequence of zygote mortality, then the addition of the dead eggs to the deficient class should at least eliminate the discrepancy. It is evident from Table 7 that this is not the case. It is therefore concluded that sperm bearing reciprocal meiotic classes do not participate equally in fertilization. This conclusion is similar to one arrived at by NOVITSKI and I. SANDLER (1957) in a different situation and will be discussed more thoroughly later. It will be noticed that three of nine duplications show the marked reduction of the XY class, and three of the four translocations show it. The significance of this escapes us at the moment. Those deletions associated with a more nearly normal recovery of the XY chromosome (0.423–0.476) apparently have the same effect as the absence of a homolog on the recovery of XY. SANDLER and BRAVER's tests of XY/0 males showed a 47.3 percent recovery of XY as opposed to 51.0 percent recovery from XY/FR-2 males. In the present experiments, 46.2 percent XY were recovered from XY/0 males. Thus it

TABLE 7

Percentage eclosion from crosses of $Y^{S}X{\cdot}Y^{L}$/Del ♂ × y/y ♀

Deletion	No. of eggs	Constitution of sperm nucleus and phenotype of progeny: XY (*y B* ♀)	Del (+ ♂)	XY/Del (*B* ♀)	0 (*y* ♂)	Eclosion: total adults / total eggs	Recovery of XY: XY+XY/Del / total adults
0	357	172	...	...	171	0.961	0.501
Dp(1;f)12	1792	583	982	0	1	0.874	0.372
Dp(1;f)18	2737	1249	1286	0	1	0.927	0.493
T(1;3)51	592	88	204	0	0	0.493	0.301
T(1;3)142	731	70	273	0	2	0.472	0.203
Dp(1;f)167	2449	888	1279	0	0	0.885	0.410
Dp(1;f)179	1140	292	797	0	3	0.958	0.267

appears that the recovery of the XY chromosome from these XY/Del males is the same as from XY/0 males even though most of the deletions do behave as homologs of the XY chromosome by the criterion of perfect disjunction.

The reduced recovery of XY-bearing sperm might be a consequence of some peculiarity of the structural relations within the XY-Dp bivalent. If so, rearrangement of the heterochromatin within the XY might result in other bivalent structures, and therefore affect this particular phenomenon. To test this idea, we investigated the effect of certain of the duplications on the recovery of $XY^{L}{\cdot}Y^{S}$ (PARKER 1954), in which the distribution of the heterochromatic elements is quite different from that in $Y^{S}X{\cdot}Y^{L}$. The data from these tests, presented in Table 8, are apparently not different from those given in Table 6. In other words, there is no apparent effect of the distribution of the heterochromatin within the XY chromosome on its meiotic behavior in combination with a duplication. It should be pointed out, however, that the heterochromatin of these two chromosomes is probably the same, and derived from the Y chromosome for a considerable distance on either side of the centromere.

TABLE 8

Progeny of $XY^{L}{\cdot}Y^{S}$, y^{2} su-w^{a} w^{a}/*Del* ♂ × y w/0 ♀

Deletion	Constitution of sperm nucleus and phenotype of progeny: XY (y^{2} su-w^{a} w^{a} ♂)	Del (*w* ♀)	XY/Del (su-w^{a})w^{a} ♂)	0 (*y w* ♀)	Recovery of XY: XY+XY/Del / total
0	3733	....	..	4367	0.461
Dp(1;f)18	2526	3613	1	23	0.410
T(1;?)42	83	190	0	25	0.279
T(1;3)51	650	1279	2	14	0.335
Dp(1;f)122	2139	3275	4	6	0.395
T(1;3)142	339	1732	9	117	0.158
Dp(1;f)179	1420	2722	5	28	0.341

Some data gathered in the process of testing the deletions for linkage with autosomes provide additional information on the observed unequal recovery of homologs. XY, *y B*/0; *Cy*/+; *Cx, D*/+ males were crossed to attached-X/Del females, and *B* sons (XY/Del) of three types (*Cy; Cx,D;* and *Cy;Cx,D*) were selected from the progeny and crossed to *y* females to test for linkage between y^+ and *Cy* and/or *D*. The data from combinations, some of which have previously given reduced recovery of the XY, are presented in Table 9. It can be seen that in the presence of *Cy* the recovery of XY from XY/Dp(1;f)179 and XY/T(1;3)51, but not from XY/T(1;3)142, becomes more nearly normal. Furthermore, Dp(1;f)167 fails to affect the recovery of the XY in the presence of *Cy* and/or *D*, whereas in the original tests its effect was considerable. These and other data suggest very strongly that the recovery of XY is influenced by the autosomal constitution. A further indication of this possibility is that whereas Dp(1;f)112 did not give a particularly low recovery of XY in the original experiments, it gave only 37.3 percent recovery in the egg count data presented in Table 7; also, statistical examination of the original data indicates culture-to-culture heterogeneity in the recovery of the XY chromosome (in some lines at least), giving in some cases normal recovery and in others a characteristically lowered recovery of XY.

The effect of some of the deletions on the recovery of the X and the Y chromosomes from X/Y/Del males has been measured. Although these data are preliminary, they are sufficient to show that recovery of Y-bearing classes may be

TABLE 9

The effect of the autosomal constitution of the parental male on the results of crosses of $Y^SX{\cdot}Y^L$/Del ♂ × y/y ♀

Deletion	Autosomal constitution of parental male	Constitution of sperm nucleus and phenotype of progeny			
		XY	Del	XY/Del	0
		y B ♀	+ ♂	*B* ♀	*y* ♂
T(1;3)51	*Cy*/+	155	186	0	2
	Cx, D/+	86	239	1	0
	Cy/+; *Cx,D*/+	87	124	1	0
Dp(1;f)52	*Cy*/+	147	168	0	0
	Cx,D/+	46	44	0	0
	Cy/+; *Cx,D*/+	59	62	0	0
Dp(1;f)122	*Cy*/+	296	264	0	0
	Cx,D/+	87	92	0	0
	Cy/+; *Cx,D*/+	191	184	0	0
T(1;3)142	*Cy*/+	63	292	1	4
	Cy/+; *Cx,D*/+	88	248	0	1
Dp(1;f)167	*Cy*/+	115	115	0	0
	Cx,D/+	112	102	0	0
	Cy/+; *Cx, D*/+	65	42	0	0
Dp(1;f)179	*Cy*/+	317	251	1	0
	Cx,D/+	31	114	0	0

Data from a selected sample of deletions.

reduced. For example, among the progeny of X/Y/T(1;3)51, 51.8 percent carry the X, 48.6 percent carry the translocation and 29.9 percent carry the Y; among the progeny of X/Y/Dp(1;f)167 males, 50.9 percent carry the X, 45.3 percent carry the duplication and 31.4 percent carry the Y. It will be noticed that in neither of these cases is any chromosome recovered in appreciably more than half of the progeny; consequently, it seems unlikely that the reduced recovery of the Y chromosome from X/Y/Del males results from impaired function of Y-bearing sperm. The data, however, can be explained by meiotic loss of the Y chromosome.

DISCUSSION

The progeny of males carrying an attached XY chromosome and certain free heterochromatic duplications derived from the X chromosome is sometimes characterized by a high incidence of duplication bearing individuals and a correspondingly low incidence of XY bearing individuals (Table 6). That this inequality in the recovery of homologs is not associated with zygote mortality can be easily demonstrated by egg counts (Table 7). We have concluded from these observations that homologous chromosomes are not recovered in *functional sperm* in the same proportion that exists in the cells of the parental male (i.e., 1:1). If a premeiotic phenomenon were the basis of the observations, then the aberrant proportions of XY to Del recovered in the mature sperm would have to exist in the primary spermatocytes. To shift the proportion of homologs in favor of the deletions, one might postulate the existence of some proportion of spermatocytes of genotype XY/Del/Del, Del/0 or Del/Del. The first of these, however, would exhibit normal recovery of the XY, and the second and third would be nullo X and would probably not survive. Consequently, it seems likely that the shift in ratio occurs between the onset of meiosis and fertilization. Generally speaking, there are two hypotheses that might be advanced to account for the observed unequal recovery of homologs: One is that the four spermatids give rise to uniformly functional sperm, but that chromosome behavior during meiosis is abnormal, such that one homolog becomes included in less than half (e.g., meiotic loss) or more than half (defined as meiotic gain) of the spermatids; the other is that each homolog is included in half the products of the meiotic divisions, but that these products—secondary spermatocytes or spermatids—produce sperm that do not function uniformly in fertilization (defined as gamete dysfunction).

Meiotic loss is visualized as the exclusion of a chromosome from both daughter nuclei of a meiotic division, which exclusion does not affect the ability of either nucleus to develop into a functional gamete, and has been correlated with univalence of the lost chromosome (SANDLER and BRAVER 1954). Exclusion could be the consequence of such factors as disintegration of the chromosome, failure of the chromosome to replicate, failure of the chromosome to become included on the spindle, or lagging. The reasons that meiotic loss cannot account for the observations described in the preceding paragraph are enumerated in the section on results. As an example of meiotic gain, the phenomenon of sex ratio in *Drosophila pseudoobscura* (GERSHENSON 1928) comes to mind. STURTEVANT and DOBZHAN-

SKY (1936) showed that in males of this and related species, which carry a certain sex-linked gene, there is commonly an extra replication of the X chromosome in the primary spermatocyte, such that four rather than two chromatids are apportioned among the four spermatids. In these spermatocytes there is a simultaneous failure to congress and subsequent disintegration of the Y chromosome (meiotic loss) with the result that nearly 100 percent of the sperm carry the X but not the Y chromosome.

For gamete dysfunction to be detectable, alternative gametic types must function unequally in fertilization. Thus if one gametic type functions in fewer, another must function in more than its share of fertilizations; consequently, the chromosomes in the complement of the latter gametic type will be recovered preferentially among the resultant zygotes. Unless this is a null complement, e.g., nullo X, nullo Y, some chromosomes will be recovered in more than half of the progeny. This provides a nearly absolute distinction between meiotic loss and gamete dysfunction as explanations of any case of unequal recovery of homologs. Meiotic loss can never result in the recovery of a homolog in more than half the zygotes, whereas, with the one exception just mentioned, gamete dysfunction must result in the recovery of a homolog in more than half the zygotes. Phenomena that can be described as meiotic gain are similar to gamete dysfunction by this criterion.

NOVITSKI and I. SANDLER (1957) published observations very similar to those presented here on the genetic behavior of $T(1:4)B^s$ during spermatogenesis. $T(1;4)B^s$ is a reciprocal translocation in which the X chromosome is broken immediately to the left of *B* and the fourth chromosome is broken distal to *ci*. They have shown that the part of the X with the fourth centromere ($=X^D$) regularly separates from the fourth chromosome, whereas the proximal portion of the X, with the tip of chromosome 4 appended ($=B^s$), regularly separates from the Y chromosome. Homologs are not, however, recovered equally frequently from either bivalent, and egg counts have shown that these inequalities cannot be explained by zygote mortality. Furthermore, the probability that a particular homolog (e.g., chromosome 4) will be recovered from one bivalent multiplied by the probability that a particular homolog (e.g., the Y chromosome) will be recovered from the other bivalent is exactly equal to the probability that they will be recovered together (Y;4). The independence in the recovery of homologs from the two bivalents, as demonstrated by this algebraic cross check, places certain limitations on the possible explanations of the observed inequalities, both for the case of $T(1;4)B^s$, and, by extrapolation, in the cases of XY/Del also. It says, in fact, that the frequency with which a homolog is recovered is independent of the genotype of the gamete in which it is recovered, thus rendering phenomena such as gamete mortality or preferential fertilization unlikely explanations of the observations. NOVITSKI and SANDLER have pointed out that the observation that gametes with perfectly normal complements (i.e., Y and 4) are recovered with reduced frequencies is also incompatible with this type of explanation. The data presented in the present report demonstrate that the same gametic type is re-

covered with a reduced frequency in some crosses but not in others, and that it is in fact the nondeletion-bearing class (i.e., XY); this provides additional evidence that the recovery of a homolog is independent of the gametic genotype.

To account for their observations, NOVITSKI and SANDLER have suggested that some of the products of spermatogenesis are regularly nonfunctional and that there is, in addition, non randomness in the orientation of the particular bivalents of a T(1;4)B^s heterozygote at first meiotic metaphase that results in the unequal inclusion of homologous chromosomes in the functional gametic nuclei, thus allowing detection of the initial asymmetry in the production of functional sperm. Independent orientation of the bivalents of T(1;4)B^s at first meiotic metaphase provides an explanation of the precise algebraic cross check mentioned in the preceding paragraph. Their hypothesis has the virtue of saying that gamete dysfunction is an attribute of the products of normal spermatogenesis, and the genotype of the male affects only the distribution of homologs to those products. As they recognize, however, it does require postulation of two independent phenomena; (1) regular dysfunction of some of the products of spermatogenesis and (2) preferential orientation of a bivalent at the first meiotic metaphase, neither of which has been demonstrated to occur in Drosophila; it might be noted, however, that orientation of asymmetric dyads does occur in the secondary oocytes of Drosophila (NOVITSKI 1951) and that, as with every case reported here, it is always the smaller element that becomes included in the functional gamete nucleus.

It seems quite likely that the phenomenon that occurs independently in the two bivalents of T(1;4)B^s males is occurring in certain of the XY/Del bivalents described in the present communication. Observation of the grossly unequal recovery of the XY versus the deletion seems to depend on some peculiarity in the nature of the bivalent (perhaps the inequality in the mass of the homologs suggested by NOVITSKI and SANDLER) and on the autosomal constitution. It may in fact be that if the autosomal requirements were understood, a wide variety of bivalents might be made to exhibit unequal recovery of homologs. The notion of gamete dysfunction provides alternative explanations of the results, including the algebraic cross check, under consideration. Most of them require that the genotype determine that specific meiotic products be nonfunctional rather than which homologs become included in meiotic products that are nonfunctional independent of the genotype. For example, one could postulate a strictly meiotic phenomenon in which, when the two chromosomes of a bivalent separate at the first division, one of them, with a probability related to events transpiring in the primary spermatocyte, in some way induces an abortive second meiotic division, or interferes with spermiogenesis, such that that particular product is recovered in fewer than half, whereas its homolog is recovered in more than half, of the functional sperm. It may be further postulated that such a phenomenon operates independently on two bivalents in a primary spermatocyte. Such a description fits aspects of a case described in Nicotiana by CAMERON and MOAV (1957). They found that the presence, in the microsporocytes of diploid *Nicotiana tabacum*, of

a particular alien chromosome derived from *Nicotiana plumbaginifolia* results in the early abortion of all microspores that do *not* carry the alien chromosome but do carry a full complement of *N. tabacum* chromosomes. These results resemble those obtained from XY/Del males in that the class of gametes that do not receive the abnormal element are recovered with reduced frequency. Finally, a model based on meiotic gain will account for the non random recovery of homologs; a phenomenon similar to that of sex ratio in *D. pseudoobscura* could be the basis of the behavior of XY/Del bivalents, and operating independently in two bivalents, could yield results similar to those observed in $T(1;4)B^s$ males. This is, of course, not an exhaustive list of alternatives to the hypothesis of NOVITSKI and SANDLER, nor has any of these alternatives a particular virtue that makes it preferable to their hypothesis. They are presented to illustrate the fact that the data presented are consistent with several models.

One final point should be made. It seems reasonably clear from the arguments presented by NOVITSKI and I. SANDLER and from the arguments presented here that the unequal recovery of homologs from males of certain constitutions is not caused by a reduced fitness of mature gametes, and does, therefore, appear to be related to the meiotic mechanics in these males. If this is indeed the case, then this phenomenon, whatever its mechanical basis, would be classified as an instance of meiotic drive (DUNN 1953; SANDLER and NOVITSKI 1957)—that is, this represents a case in which gene frequency could be drastically influenced by some meiotic phenomenon.

SUMMARY

The disjunction of a series of X chromosome deletions (constructed by deleting, with X irradiation, all but a negligible amount of the euchromatin of the X and a variable amount of the proximal heterochromatin) was studied in X/X/Del females, attached-X/ Del females, and attached-XY/Del males. With one interesting exception, the data agree with the notions of GERSHENSON (1940) which are outlined in the introduction of this paper. The exception was found in the results from matings of males carrying the attached XY chromosome and certain of the deletions. Among their progeny the deletion is recovered in considerably more than half of the individuals whereas the XY is recovered in considerably less than half. The following points have been established: (1) Egg counts have shown that this is not zygote mortality. (2) The fact that the deletions are recovered in excess of 50 percent of the progeny eliminates meiotic chromosome loss as the explanation of the discrepancy. (3) Evidence is presented indicating an autosomal influence on this phenomenon. Finally, it has not yet been possible to determine the physical basis of these results. However, it appears that whatever the physical basis, this phenomenon could cause meiotic drive in a population.

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GENETIC STUDIES OF SEVERAL CHARACTERS IN COLIAS EURYTHEME (LEPIDOPTERA, PIERIDAE)

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REMINGTON (1954) recently reviewed the previous studies on genetics of Colias. The sex-limited "alba" gene, which controls female dimorphism, has been studied in *C. eurytheme* Boisduval, *C. philodice* Latreille, *C. christina* Edwards, *C. myrmidone* Esper, and in *C. erate poliographus* Motschlsky. Besides this well studied gene, inheritance of the following genes has been investigated: "blue-green" and "olive-green" larval color in *C. philodice* (GEROULD 1926); blue-green larval, pupal and adult eye color, recessive white male and female wing color, and egg sterility and food plant sterility. (HOVANITZ 1944); discal spot color on the hind wings in *C. philodice*, *C. eurytheme*, and *C. erate poliographus* (KOMAI and AE 1953; REMINGTON 1955; DEAN 1956; AE 1957); and inheritance of orange and yellow ground coloration in the hybrids between *C. eurytheme* and *C. philodice* (GEROULD 1943; HOVANITZ 1944; REMINGTON 1954). AE (1956) also studied the genetics of hybrids between *C. eurytheme* and *C. interior* Scudder.

As FORD (1945) has pointed out, wild butterflies carry many recessive genes in heterozygous condition, and sib matings in the laboratory produce some recognizable recessive homozygotes.

Certain new hereditary characters in *C. eurytheme* were found by means of sib matings, and their genetics has been investigated. Two of these characters were transmitted into the hybrids between *C. eurytheme* and *C. philodice*. The collection of Colias at the American Museum of Natural History, New York, and most of the specimens of GEROULD's genetic studies (1923, *et al.*) at the Peabody Museum of Natural History at Yale University also have been examined. The results are presented and discussed here.

MATERIALS AND METHODS

C. eurytheme orange female, P–62, which was collected at Yale University by the writer, carried the several hereditary characters described below. The progeny from one other *C. eurytheme* and one *C. philodice* in New Haven, and from two *C. eurytheme* in Jackson, Mississippi, were also used in this study.

The rearings were carried on in a rearing room of the Josiah Willard Gibbs Laboratory, at Yale University. The room was kept at 80°F, and fluorescent light illumination was used 14 hours per day. Humidity was kept low and approximately constant in the room.

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Eggs were obtained from females in glass lamp chimneys on potted food plants, placed under the sun or near an incandescent lamp. The larvae were reared in insect cages about 10 inches cubed, of which all four sides were netting and the top was glass. Vetch (*Vicia villosa*) was used as the sole larval food. Matings were obtained in cages near the laboratory window, under either direct sunlight or under strong fluorescent light in the rearing room. A mixture of one part of clover honey and two parts of water was used as food for the adults. The butterflies were stored at 60°F in a dark room when they were not being used for mating or egg laying.

During the rearing, a large number of larvae or pupae often died of diseases. Their symptoms coincide precisely with the description by STEINHAUS (1949) of polyhedroses by virus infection in Colias. Therefore, these losses are referred to simply as virus diseases in this paper, although microscopic tests were not performed.

An individual designation was given to each wild female which became the mother of a brood, and to all laboratory reared males and females. The individual designation of a female was also used as the designation of its brood. For instance, the wild *C. eurytheme* female, P–62, produced Brood P–62, and one of the females of this brood, P–62–9, mated with one of its sibs, P–62–2, and produced Brood P–62–9.

RESULTS

"Black-veined" character

On 20 December 1956, one *C. eurytheme* mutant male emerged from a stock from female P–62 which had been continuously inbred by sib matings. Its wing veins on the upper side were almost completely black as in the Monarch butterfly (*Danaus plexippus* L.). The ancestors of this butterfly were carefully examined and further matings and rearings were carried on in order to clarify the genetics of this new mutant (Figure 1).

Figure 2 shows the analysis of ancestors and sibs of the above "black-veined" mutant, P–62–9–7–2, which is one of the four "black-veined" mutants of Brood P–62–9–7. The expression of blackness of veins varied extensively. All veins were black on the upper sides of both wings in the most prominent ones. In some individuals veins of the upper side of the hind wings only were clearly black. Only traces of blackness were found in veins of the central portions of the hind wings in other individuals. In many females in this strain and others, there is a tendency for black lines to extend along veins from the border to the base of the wing. However, no correlation of this female condition with male "black-veined" was recognized.

Brood P–62–9–7 was a very productive brood, and a total of six sib matings and three other kinds of matings were obtained. Some males were mated two or three times, and two females were mated twice. Although some of the matings were completely sterile, a large number of progeny, which included many "black-veined" mutants, was obtained. Table 1 shows the direct progeny from

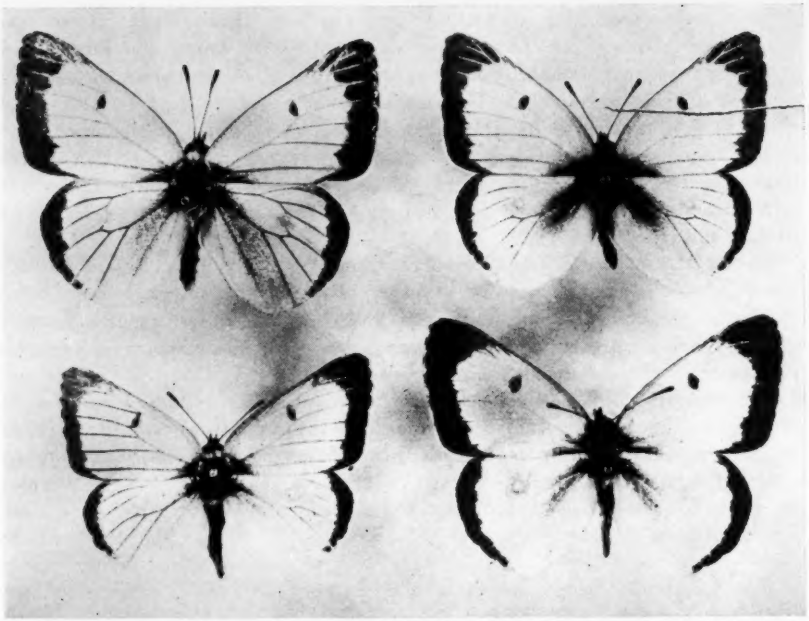

FIGURE 1.—Three "black-veined" mutants of *Colias eurytheme*. Normal male is shown in lower right hand corner.

TABLE 1

F_1 from females of Brood P–62–9–7 (B)

Brood	Male parent	Male parent phenotype	Offspring				
			Females	Males			Total
				Bv	Tr	No	
B–3	B–4	No	7	0	0	3	10
B–5	B–12	No	3	1	0	3	7
B–9	B–1	Tr	11	3	1	4	19
B–10	P–76–8	No	10	0	0	12	22
B–13	B–4	No	0	3	0	1	4
B–18	B–15	No	4	0	1	8	13
B–21	B–9–5	Bv	1	0	3	1	5

the above matings. Sterile matings and other matings which failed to produce adult progeny were excluded from the table. Brood P–62–9–7 was designated as Brood B in this table and hereafter.

Figure 3 shows a genealogy of a part of the "black-veined" strain which produced an all "black-veined" brood. Figure 4 shows a genealogy of another part of the "black-veined" strain which included one *C. philodice* male, P–76–8, and

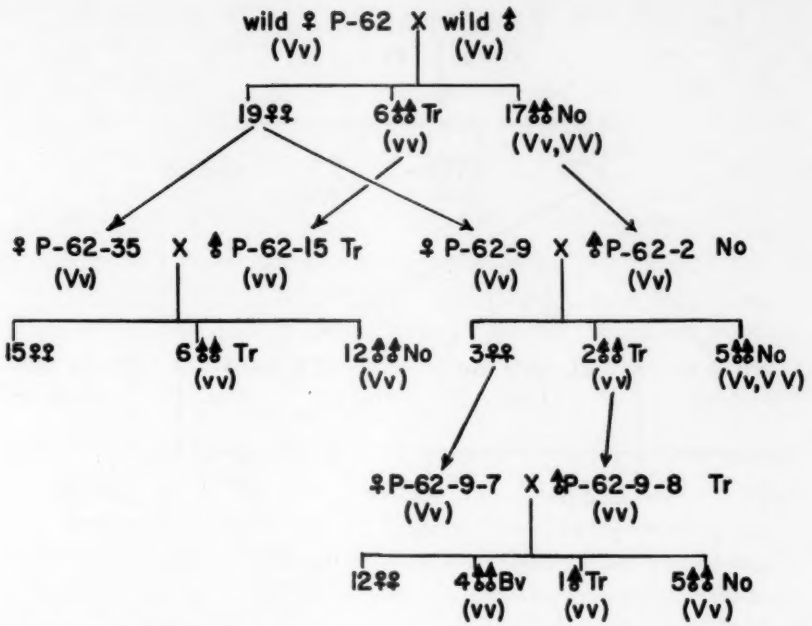
wild ♀ P-62 X wild ♂
(Vv) (Vv)
19♀♀ 6♂♂ Tr 17♂♂ No
(vv) (Vv,VV)
♀ P-62-35 X ♂ P-62-15 Tr ♀ P-62-9 X ♂P-62-2 No
(Vv) (vv) (Vv) (Vv)
15♀♀ 6♂♂ Tr 12♂♂No 3♀♀ 2♂♂ Tr 5♂♂No
(vv) (Vv) (vv) (Vv,VV)
♀P-62-9-7 X ♂P-62-9-8 Tr
(Vv) (vv)
12♀♀ 4♂♂Bv 1♂ Tr 5♂♂ No
(vv) (vv) (Vv)

FIGURE 2.—Genealogy of a ""black-veined" strain (AE I). **No**-normal, **Tr**-trace black-veined, **Bv**-Black-veined.

an unrelated *C. eurytheme* female, P–78–4–102, which was from Mississippi stock.

The writer found two wild *C. eurytheme* males which show "black-veined" in trace expression in the collection of the American Museum of Natural History. One was collected by LICK BROOK at Ithaca, N.Y., on 19 August 1928. Another was collected by H. J. SANFORD at Manahawkin, Ocean County, N.J., on 15 October 1948.

The writer also examined most of the specimens reared by J. H. GEROULD in his genetic studies of *C. eurytheme* and *C. philodice* (1923), now deposited in the Peabody Museum of Natural History at Yale University. Two strains of *C. philodice* proved to include "black-veined" males. Figures 5 and 6 show their genealogies. No difference was found in expression of "black-veined" in *C. eurytheme*, *C. philodice* and hybrids between them.

These studies clearly indicate that "black-veined" is a hereditary character, which is recognized only in males. Phenotypes vary in intensity of expression of the "black-veined" character. The most probable interpretation for the trait is that it is a single recessive. Table 2 shows results obtained from matings in which the genotypes of parents were fairly certain, as derived from their ancestors or progeny. The mating between a strongly "black-veined" male,

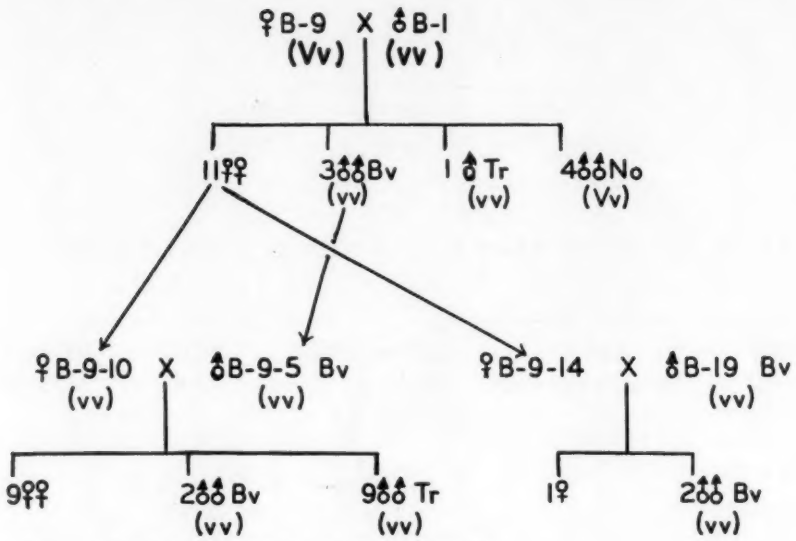

FIGURE 3.—Genealogy of a "black-veined" strain (AE II).

TABLE 2

F_1 of Colias crosses of "black-veined" strain

Brood	Male parent	Male offspring	
		Normal	"black-veined"
	a. *Vv* × *Vv*		
P–62	wild	17	6
1909 2w	1a[6]	13	3
1910 a	wild	12	2
1910 e	a[7]	51	4
	b. *Vv* × *vv*		
P–62–35	P–62–15	12	6
P–62–9–7 (B)	P–62–9–8	5	5
B–9	B–1	4	4
B–21	B–9–5	1	3
B–10–17–4	B–3–4–19	1	6
	c. *VV* × *vv*		
P–78–4–102	B–3–4–26–8	23	0
	d. *vv* × *vv*		
B–9–10	B–9–5	0	11

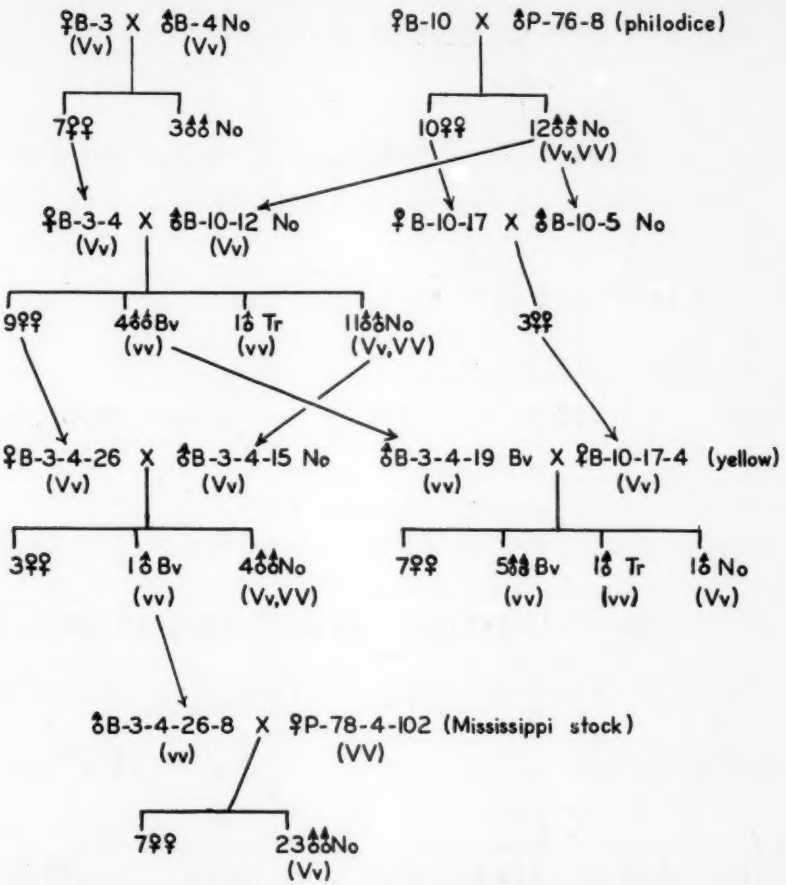

FIGURE 4.—Genealogy of a "black-veined" strain (AE III). B = P-62-9-7.

B–3–4–26–8, and an unrelated (to "black-veined" strain) female, P–78–4–102, produced 23 normal males (Figure 4, Table 2). This suggests complete dominance of the wild type allele of the "black-veined" locus. It is difficult to interpret the ratio of 51 normal : four "black-veined" (Figure 6 & Table 2) in GEROULD's brood 1910e. Possible explanations include a reduced penetrance of genes for "black-veined" in homozygous individuals, or a low viability of the homozygotes. The observed 13 : 3 and 12 : 2 ratios for theoretical 3 : 1, and 12 : 6 for presumed 1 : 1 ratio fit the above interpretation. Furthermore, female B–21 and B–10–17–4 could have been recessive homozygotes, judging from their ancestors, on the assumption that one normal male in each brood was a

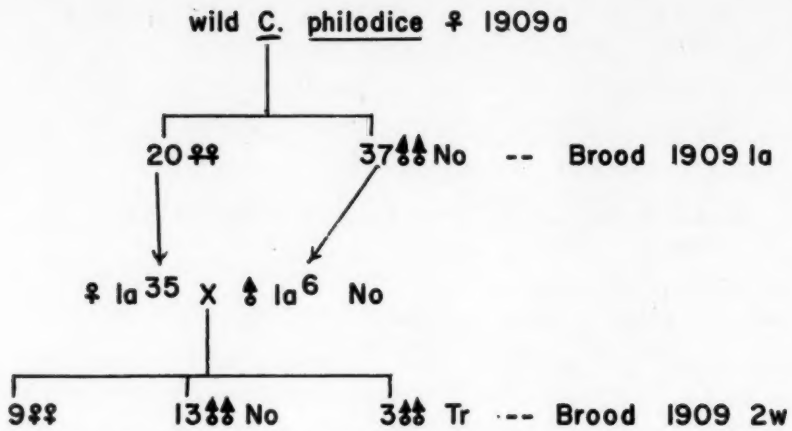

FIGURE 5.—Genealogy of a "black-veined" strain (Gerould I).

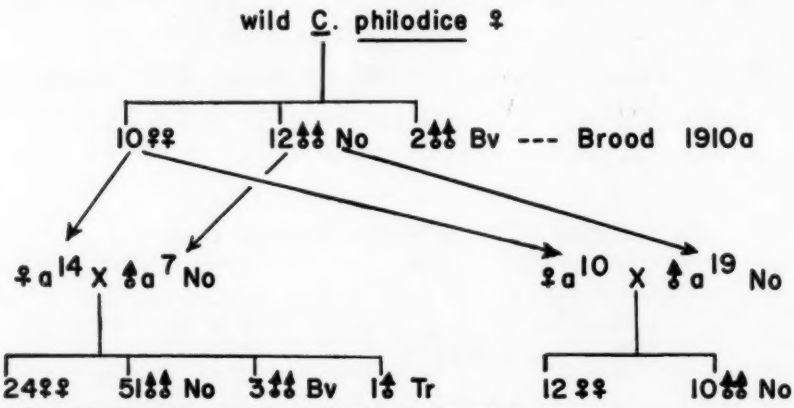

FIGURE 6.—Genealogy of a "black-veined" strain (Gerould II).

recessive homozygote, which had its normal expression decreased by a low penetrance. Therefore, 1 : 3 and 1 : 6 ratios for 1 : 1 do not necessarily indicate an excess of recessive homozygotes, but rather a modification from 0 : 1 ratio.

Glueless female

Four females, out of nine in Brood B which laid eggs, failed wholly or partially to glue their eggs on the leaves of food plants. This tendency was transmitted to their progeny extensively. Figure 7 shows its genealogy. *G* indicates glue, namely individuals which glued their eggs on the leaves, and *g* indicates glueless (wholly or partially). Females which were not examined were excluded from

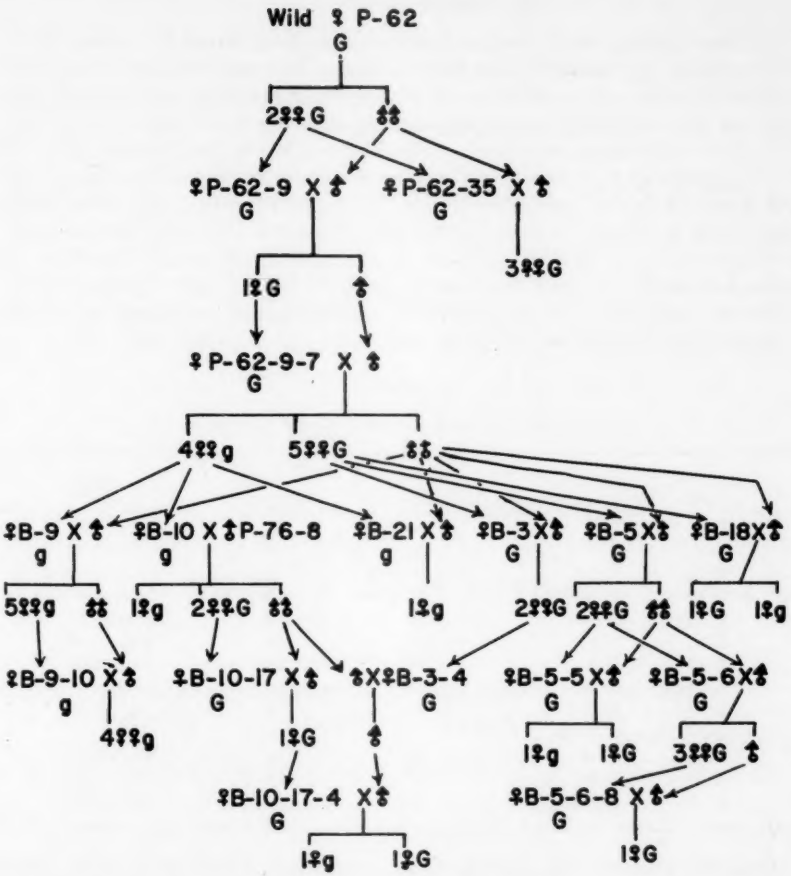
Wild ♀ P-62
G
2♀♀G ♂♂
♀P-62-9 X ♂ ♀P-62-35 X ♂
G G
3♀♀G
1♀G ♂
♀P-62-9-7 X ♂
G
4♀♀g 5♀♀G ♂♂
♀B-9 X ♂ ♀B-10 X ♂P-76-8 ♀B-21 X ♂ ♀B-3 X ♂ ♀B-5 X ♂ ♀B-18X ♂
g g g G G G
5♀♀g ♂♂ 1♀g 2♀♀G ♂♂ 1♀g 2♀♀G 2♀♀G ♂♂ 1♀G 1♀g
♀B-9-10 X ♂ ♀B-10-17 X ♂ ♂X♀B-3-4 ♀B-5-5 X ♂ ♀B-5-6X ♂
g G G G G
4♀♀g 1♀G 1♀g 1♀G 3♀♀G ♂
♀B-10-17-4 X ♂ ♀B-5-6-8 X ♂
G G
1♀g 1♀G 1♀G

FIGURE 7.—Genealogy of a "glueless" strain. *G*-glue, *g*-glueless.

this genealogy. Some wild females which were kept at 60°F in a dark room for about one month or more also failed to glue eggs during the experiments. However, glueless females of this genealogy had been kept in the 60°F room for only a few days or less before they failed to glue eggs. Therefore, its hereditary basis is fairly clear. Although the data are not sufficient to justify a discussion of genes conditioning this character, the most probable interpretation is a six-limited, single dominant gene, because this character seems to carry through males and crossing between glueless female B–10 and unrelated *C. philodice* male produced a glueless female and two glue females.

Male sterility

Thirteen matings were obtained from seven males of Brood B. Table 3 shows the results of egg fertility from these matings. Two males mated three times and the other two males mated twice. Five matings produced only infertile eggs (did not turn red), and one mating produced only one fertile egg out of 44 laid. The above six matings were from males B–2 and B–14. Two females, B–5 and B–13, mated with B–2, later mated again with males respectively B–12 and B–4 and produced fertile eggs. One female, B–18, mated with B–14, later mated again with B–15 and produced fertile eggs. Figure 8 illustrates the situation. It is clear that the sterilities of these six matings are the result of sterility of males B–2 and B–14. Since the above seven males were all sibs and the progeny of the two continuous sib matings, these sterilities could have been the results of one or two pairs of recessive genes.

TABLE 3

Egg fertility of mates of Brood P–62–9–7 (B) males

Parents		Eggs laid	Fertile eggs	Egg fertility percent
Males	Females			
B–1	B–9	184	142	77.2±3.09
B–2	B–5	21	0	0.0
B–2	B–13	11	0	0.0
B–2	P–70–6–9	31	0	0.0
B–4	B–3	124	99	79.8±3.61
B–4	B–13	135	54	40.0±4.22
B–12	B–5	46	36	78.3±6.08
B–14	B–18	21	0	0.0
B–14	B–16	44	1	2.3±2.26
B–14	B–9–12	17	0	0.0
B–15	B–18	78	70	89.7±3.44
B–19	B–22	56	13	23.3±5.65
B–19	B–9–14	50	18	36.0±6.79

From the rearings and matings of the progeny of the above matings, large numbers of completely sterile matings were obtained, mainly by continuous sib matings (Table 4). Although it was not determined whether females or males were responsible for these sterilities, many of the above sterile matings could be the results of the same gene(s) which caused the sterilities of B–2 and B–14.

Reductions of fertility and hatchability

On the other hand, the general reduction in fertility progressed with continuation of sib matings (Table 5). The egg fertility fluctuated extensively among the different matings within the same generation, but steadily declined with generation after generation. This could be interpreted as homozygosity of many recessive genes which as polygenes reduce egg fertility.

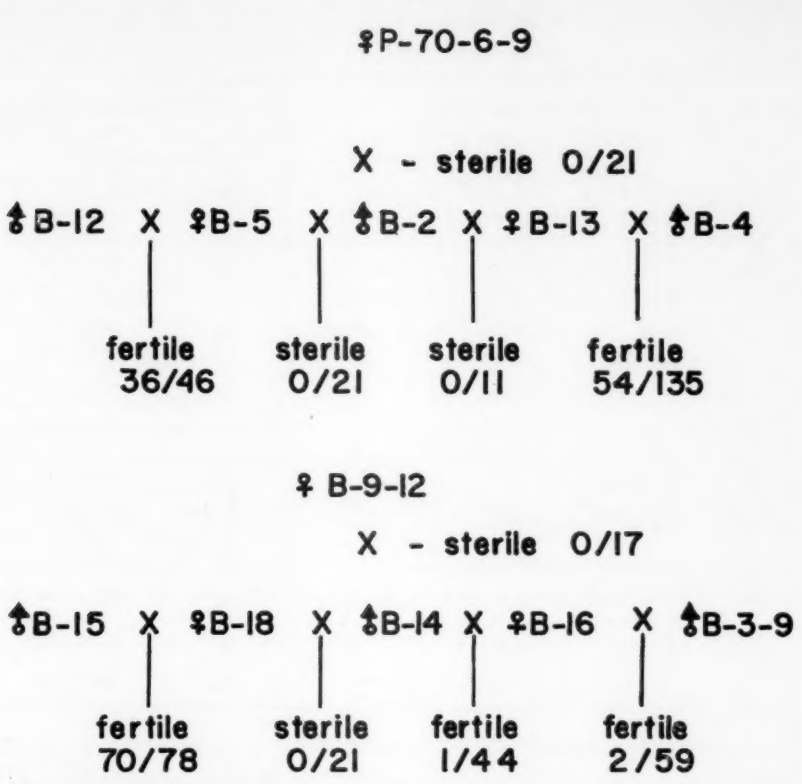

FIGURE 8.—Genealogy of sterile males, B-2 and B-14.

TABLE 4

Ratio of number of crosses of egg fertility less than five percent to total number of crosses in continuous sib matings from female P-62

Generation	F_1	F_2	F_3	F_4	F_5	F_6	F_7
Ratio	0/1	0/2	2/4	4/10	2/7	7/10	1/1

The reduction of egg hatchability seems to follow the same line as the above. Although the hatchability was not recorded in detail, no reduction was observed in the first and second generations of sib matings, about 75 percent hatch was observed in third, and a fluctuation between 75 to 50 percent hatch in the fourth, and between 75 to 25 percent hatch in fifth and sixth were observed. This could also be polygenic.

TABLE 5

Reduction in egg fertility in continuous sib matings from female P–62

Generation	Mother	Father	Eggs laid	Fertile eggs	Egg fertility percent
F_1	P–62 (wild)	wild	43	43	100.0
F_2	P–62–9	P–62–2	37	37	100.0
F_3	P–62–9–7	P–62–9–8	118	112	94.9±2.03
F_4	B–3	B–4	124	99	79.8±3.61
	B–9	B–1	184	142	77.2±3.09
	B–5	B–12	46	36	78.3±6.08
	B–18	B–15	78	70	89.7±3.44
	B–13	B–4	135	54	40.0±4.22
	B–22	B–19	56	13	23.3±5.65
		Subtotal	623	414	66.5±1.89
F_5	B–9–9	B–9–1	29	8	27.6±8.30
	B–9–4	B–9–3	16	6	37.5±12.1
	B–9–10	B–9–5	205	94	45.9±3.48
	B–5–5	B–5–2	104	69	66.3±4.64
	B–5–6	B–5–1	107	89	83.2±3.62
		Subtotal	461	266	57.7±2.30
F_6	B–9–10–18	B–9–10–1	203	51	25.1±3.04
	B–9–10–19	B–9–10–9	15	11	73.3±16.9
	B–5–6–8	B–5–6–2	186	92	49.5±3.66
		Subtotal	404	154	38.1±2.42

DISCUSSION

Three recessive characters, namely, "black-veined", male sterility, and a reduction in egg fertility and hatchability, were found from one wild *C. eurytheme female*, P–62. This may be an indication of extensive heterozygosity in wild Colias. The "black-veined" gene seems to be common in nature. However, a homozygote of this gene seems to have a low viability or penetrance in nature, and a higher viability or penetrance in the laboratory. Only two "black-veined" males were found among a very large collection of *C. eurytheme* and *C. philodice* in the American Museum of Natural History, and three "black-veined" strains and a large number of "black-veined" males were obtained from the rearings by GEROULD and the writer. This gene seems interchangeable between *C. eurytheme* and *C. philodice* without altering its phenotype. A possibility that, a slightly "black-veined" individual is a heterozygote, may not be completely denied. However, 23 normal males from a crossing between a strongly "black-veined" male and unrelated female seems to be good evidence that slightly "black-veined" individuals are not heterozygotes, but weakly expressed homozygotes. There is another possibility, that this character is polygenic in control. However, the following evidence may deny this possibility. Female,

B–10, of the "black-veined" strain, was mated to a male of an unrelated *C. philodice* stock, and three females were obtained from a sib mating of their progeny. One of them mated with a strongly "black-veined" male and produced strongly "black-veined" males in the F_1. There remains a possibility that two pairs of genes are controlling this character. But the frequent appearance of strongly "black-veined" males in different broods may reduce this possibility. One must always consider the possibility that an environmental condition has effected the "black-veined" expression. However, the conditions which the writer used seem never or very seldom to produce a phenocopy of "black-veined" from the wild type allele, because no "black-veined" appeared in a very large number of unrelated individuals (to "black-veined" strain) which were reared in the same room.

The data are not sufficient to discuss their heredity basis for other characters in detail. The most probable interpretations were stated in the previous section. Rearing conditions may reduce egg fertility or hatchability. However, it seems probable that rearing conditions which were used in these experiments were favorable enough that extensive reductions in fertility and hatchability did not take place. Long, continous sib matings were successful in the rearing room and the butterflies mated very well without help of sunlight. Many of the butterflies laid 100 to 200 eggs in a few days; facilities prevented us from obtaining more eggs. No noticeable reductions of adult sizes or pigmentations took place. When an individual obtained from a continuous sib mating was mated to a nonsib, recoveries of egg fertility and hatchability were sometimes very noticeable.

SUMMARY

1. Four hereditary characters, "black-veined", "glueless", male sterility, and a reduction of egg fertility and hatchability, were obtained from a wild *Colias eurytheme* female, P–62.

2. The "black-veined" character on the wing is a male sex-limited single recessive and has various phenotypes.

3. The above character was transmitted into the hybrids between *C. eurytheme* and *C. philodice*.

4. Two wild-caught "black-veined" *C. eurytheme* males were found from the collection at the American Museum of Natural History.

5. Two "black-veined" strains were found from specimens of *C. philodice* reared during GEROULD's genetic work.

6. The "glueless' character seems to be controlled by one pair of female sex-limited dominant genes.

7. Male sterility seems to be controlled by one or two pairs of recessive genes.

8. A reduction of egg fertility and hatchability seems to be controlled by polygenic systems.

ACKNOWLEDGMENTS

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The cooperation of the authorities of the American Museum of Natural History for allowing him to study the Colias collections is appreciated.

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CHROMOSOMAL ANALYSES OF THE GENETIC FACTORS FOR RESISTANCE TO DDT IN TWO RESISTANT LINES OF DROSOPHILA MELANOGASTER[1]

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THE data which had accumulated over a period of more than four years of experimenting with the problem of resistance to DDT in populations of *D. melanogaster* all pointed to the conclusion that resistance was inherited not as a single gene but as a polygenic complex. Numerous resistant lines had been produced by selection. In some the mean tolerance had been increased more than twentyfold. But response to selection was always slow and it was as rapid at a relatively low level of selective intensity when only the most susceptible half of the mortality distribution was eliminated, as it was when selection was more intense and only the five or ten percent of most resistant individuals were allowed to survive. Different strains responded differently to selection. Different parallel lines stemming from the same strain and selected in the same way did not respond identically. In some crosses between such lines it was quite clear that dissimilar factors for resistance were segregating in the F_2 (KING 1954, 1955a, b).

This evidence for a polygenic system as the basis of resistance was strong but it was mostly indirect and inferential. It therefore seemed advisable to try for more direct evidence by measuring separately the mean tolerance of a series of groups of flies, the flies in each group carrying one of the possible combinations of resistant and nonresistant chromosomes. The simplest way of doing this would have been to use chromosomes with dominant markers as the susceptible ones, but there were several reasons why it did not seem best to follow this procedure.

In the first place, flies carrying dominant markers, even if they are as viable as wild type flies (which they often are not), tend to behave differently. Curly-winged flies, for example, fly much less than wild type. Since all our measurements of resistance had been made by treating flies with an aerosol of DDT dissolved in tri-n-butyrin, it seemed very questionable to treat with an aerosol flies of widely different phenotypes having very different characteristics of behavior and to assume that the effective dosage would be the same. Secondly, earlier experiments had shown (KING 1955b) that in some crosses there was evidence of nonadditive interactions between genetic factors for resistance from different lines. Hence it seemed inadvisable to run tests on flies in which chromosomes from a resistant line and those from laboratory tester stocks were both

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present. In such a case we would have had no way of knowing whether the genetic material of the tester stock was interacting with the factors for resistance and if it was, in what direction and to what degree.

The material best suited to such an analysis appeared to be groups of wild type flies carrying the different possible combinations of resistant and susceptible chromosomes, the former drawn from a given resistant line and the latter from the control population from which the resistant line had been developed by selection. Such flies can be obtained by mating both susceptible and resistant flies to tester stocks carrying marked chromosomes and then making the appropriate series of matings between the resulting heterozygotes which carry marked chromosomes and known unmarked chromosomes.

MATERIALS AND METHODS

This type of analysis was carried through separately for two resistant lines—SyS-1002 and SyS-102. Both lines stemmed from a population of wild type flies descended from about two dozen individuals collected in a grocery store in Syosset, New York in July 1952. This Syosset population (designated by the symbol Sy) was maintained in the laboratory as a control, for the first year in mass cultures, from July 1953 on, in a population cage where the number of flies fluctuated between six and ten thousand. Samples from the population were tested continuously for resistance over a five year period and while there were fluctuations in LD_{50} through time, they were insignificant compared with the differences between the control and the two selected lines subjected to analysis.

SyS-1002 was set up in October, 1952. Selection was carried out by subjecting adult flies to an aerosol of DDT dissolved in tri-n-butyrin and using as the parents of the next generation several hundred flies which had survived a dose killing about 50 percent of those treated. A definite increase in resistance was apparent after about a dozen generations of selection. From this point resistance increased at a steady and more rapid rate until generation 40 when the LD_{50} had reached about 16 times that of the control. After F_{40} resistance continued to increase but at a somewhat slower rate. At F_{63} when flies were taken for the chromosome analysis the LD_{50} was about 20 times that of the control.

SyS-102 was set up in November, 1952 and selected at a higher level of intensity. The flies used as parents in this line had survived a dose of DDT killing approximately 95 percent of those treated. The number of pairs of parents varied from generation to generation from a minimum of four to a maximum of 20. At this intensity of selection there were too few offspring from the survivors to permit selection in every generation. These offspring were allowed to breed without exposure to DDT and in the following generation selection was repeated. Thus SyS-102 was selected only in alternate generations. There was no very pronounced response to selection until F_{34} when the LD_{50} reached four times that of the control. It remained at about this level until F_{50} when it suddenly jumped to 16 times. In subsequent generations it fluctuated around 20 times. Flies of F_{85} were used in carrying out the chromosome analysis.

For the purpose of making these analyses, two tester stocks were built up with dominant markers on the X, the second (II), and the third (III) chromosomes. In one of these the females carried attached-X chromosomes marked with yellow; in the other the X was Muller-5 carrying *sc*^{8} *w*^{a} *B*. In both stocks the II and the III were balanced lethals. One II was *SM-1* (KRAMER and LEWIS 1956) carrying *Cy al sp*, the other was the standard Plum (*Pm*) chromosome; one III was *Ubx*^{130} *e*^{s} (LEWIS 1952), the other Stubble bristle (*Sb*). Chromosome IV, which comprises a very small portion of the genetic material, was not followed in the analysis.

With two types of chromosomes—resistant and nonresistant—and three chromosome pairs, there are 27 different combinations possible in females. Two of these represent the resistant line and the control. Of the other 25, six have no heterozygous pairs of chromosomes and can be carried from generation to generation without producing recombined chromosomes partly of resistant and partly of nonresistant origin. The other 19 combinations are heterozygous in one or more pairs of chromosomes. By means of a mating scheme illustrated in the accompanying diagram, the six true-breeding combinations were produced. The 19 heterozygous groups were all obtained by making crosses using different combinations of the six true-breeding stocks, the resistant line itself or the control. For these 19 groups, only F_1 flies were tested so that no recombinant chromosomes were ever involved.

In the diagram (Figure 1) resistant wild type chromosomes are identified by a subscript "r" and nonresistant (susceptible) by a subscript "s". Since these

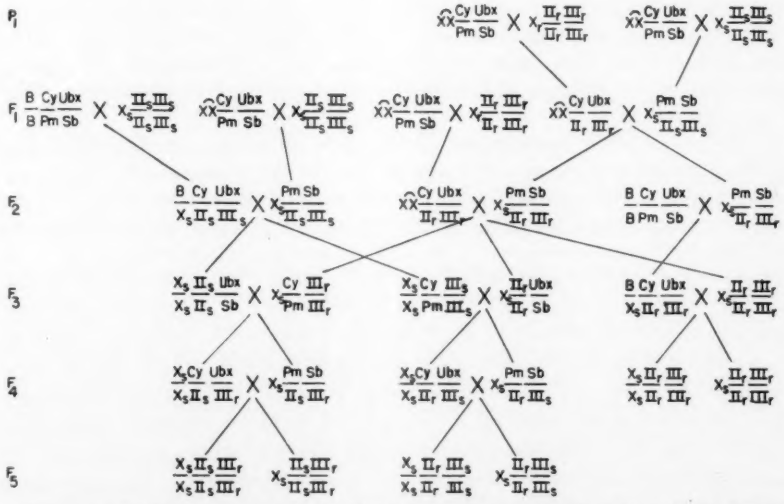

FIGURE 1.—Mating scheme used for obtaining different combinations of resistant and nonresistant wild type chromosomes.

symbols are cumbersome, we devised a simple code which will be used in the text. The nonresistant X, II, and III chromosomes are represented by 1, 2, and 3 respectively, and their resistant homologues by 4, 5, and 6. The control females are thus 11–22–33 and the resistant females 44–55–66. The females of the F_1 of a cross between these two lines would be 14–25–36. Each of the 27 combinations is for convenience identified by the karyotype of the female. The three true-breeding combinations shown as the result of the mating scheme in the diagram are, reading from left to right, 11–22–66, 11–55–33, and 11–55–66. The other three true-breeding combinations—44–22–66, 44–55–33, and 44–22–33—were obtained in a similar way, but the F_1 crosses were altered by using resistant males at the extreme left and females heterozygous for susceptible and males heterozygous for resistant chromosomes at the extreme right and by interchanging the positions of the two middle crosses.

It is well established that there are genetic differences between individual chromosomes isolated from any population. Consequently, it was desired to work with a sample array of different chromosomes for each pair drawn at random from the resistant line and from the control. This would give us, we hoped, a picture of what the chromosomes were doing in the randomly mating populations. An analysis made using a single chromosome from each pair from each population might give a very aberrant picture. To assure having such an array of different chromosomes, from 100 to 150 wild type males were mated to an approximately equal number of virgin females of the tester stocks in those P_1 and F_1 crosses where wild type males were used. These were divided among from 25 to 50 cultures, each culture having from three to four pairs of flies. When flies were collected from these cultures for use in crosses in the following generation, care was taken to collect approximately equal numbers of the phenotypes desired from each culture. This same procedure was followed throughout the mating program in order to insure the maintenance of a representative sample of different chromosomes.

In the course of the mating program the phenotypes to be used in subsequent crosses appeared in different ratios and for this reason the number of cultures per cross was varied. When a particular phenotype could be expected to appear with a low frequency, a larger number of cultures was set up. Usually 12 cultures were sufficient, but in some crosses from 20 to 30 were set up and, in two cases, 60. Each culture was set up with from two to four pairs of flies. The smallest total number of parents ever used was 18 females and 14 males divided among seven cultures. This was in the SyS-1002 series, the F_3 cross at the extreme right in the diagram.

As a result of these procedures we recovered in F_4 and F_5 wild type flies carrying almost no genetic material from the tester stocks. The *SM-1* and *Ubx* chromosomes contain complex multiple inversions and these were apparently effective in preventing recombination between tester and wild type chromosomes. No phenotypes indicating any such crossovers were ever observed. Throughout the mating program the only females ever used were those carrying these chromosomes. Since in the male there is no crossing over, males carrying *Pm* and *Sb*

chromosomes, which are less effective in preventing crossovers in the female, could be used. The Muller-5 chromosome also contains inversions but in the highly abnormal nucleus where the two large autosomes were also heterozygous for complex inversions, some crossovers between the X chromosomes occurred. These were manifested by males having w^a non-*B* eyes. Such males were discarded, but it was impossible to eliminate all recombinant X chromosomes because they could not be detected in heterozygous females. However, the total proportion of these recombinants was low. In the wild type flies which were tested for resistance, about three percent of the males had w^a eyes. The Y chromosomes also came from either the control (Sy) or the resistant line, but it was always from the source opposite to that of the X.

When the wild type flies were collected in F_4 and F_5 an effort was made again to get as large numbers as possible to insure having a reasonably good sample of wild type chromosomes. Table 1 summarizes the data on the number of females

TABLE 1

Number of wild type flies used to start stocks of the true-breeding combinations of resistant and nonresistant chromosomes and the number of cultures from which they were collected

Chromosome combination	Females	Males	Total	No. of cultures
		SyS–1002 Series		
11–22–66	274	227	501	57
11–55–33	370	278	448	59
11–55–66	25	9	34	7
44–22–66	131	117	248	24
44–55–33	113	99	212	24
44–22–33	30	23	53	15
		SyS–102 Series		
11–22–66	83	63	146	25
11–55–33	83	74	157	25
11–55–66	82	83	165	17
44–22–66	78	56	134	24
44–55–33	88	92	180	24
44–22–33	85	70	155	15

and males with which the stocks of the six true-breeding combinations of wild type flies were started. The number of cultures from which these were taken is also given. It can be seen that our minimum sample of chromosomes must have approximated two dozen and that in most cases we had considerably more.

The mating program was carried out first with SyS-1002. The P_1 crosses were made about the first of October, 1956 and the last F_5 offspring were collected about December 1. The testing of the combinations for resistance began December 17 and was finished on February 28, 1957. For SyS-102, the P_1 crosses were made about January 1, 1957 and the last F_5 offspring collected during the first week in March. The testing for resistance in the 102 series began on March 11 and was finished on May 2. Throughout the entire experiment the cultures were

kept at 25°C ±1° and the humidity in the laboratory was kept near 60 percent although there was no automatic control.

During the course of the testing program, we had to carry the six true-breeding stocks through four or five generations in order to have eggs of each stock available when they were needed. This was done by keeping the flies of each stock in a small population cage (4¼″ × 3½″ × 6″) and collecting eggs laid on food cups placed in the cages. The first generation of each stock was limited to the number of flies collected as shown in Table 1, but in all subsequent generations the number of flies in a cage was about 1000. Eggs from the control flies were obtained by placing sample cups in the control population cage. Eggs from the resistant lines were obtained from the populations which were still being subjected to selection. In neither line was there any substantial increase in resistance while the analysis was going on. In order to obtain eggs heterozygous for resistant and nonresistant chromosomes, pairs of cultures of the appropriate true-breeding, control or resistant lines were made up, 500 virgin females were collected from one set and 500 males from the other. These flies were then placed in a small population cage and the heterozygous eggs collected. To produce 11–25–36, for example, females from 11–55–66 were mated to control males (11–22–33).

Approximately 20,000 flies of each combination were raised in 100 culture bottles seeded with eggs collected in a population cage. Twenty-five bottles were seeded on each of four successive days. Fourteen days later the resulting flies were subjected in groups of 1000 to the aerosol of DDT. A series of time doses was selected for each combination to give a spread in mortality of from around ten percent to around 90 percent. The resulting dose-mortality figures were then plotted on a log probit scale and the LD_{50} calculated by the maximum likelihood method as described by Finney (1952). Thus a log LD_{50} for every one of the 27 chromosome combinations was obtained. For the control the figures were calculated from tests run on the control flies during the period when the other combinations were being tested. For the resistant lines the figures were those of the generation which had been used in the P_1 and F_1 crosses of the mating program.

Throughout the entire selection program male and female flies were treated together and mortality figures for the two sexes were not kept separately. For a number of technical reasons it was not practical to do otherwise. This same procedure was followed in the chromosome analysis. This means that all log-probit regression lines were based on composites of two mortality distributions with different means, one for males and one for females, for in all cases males as a group are less resistant than females. This has caused the variance of the mortality distribution and the error of the LD_{50} to be higher than they would have been if the two sexes had been counted separately. In the case of those chromosome combinations where the females were heterozygous for the X—all the 14's—there were two possible ways to make the cross. If females 44 were used, all males would have had resistant X chromosomes; if females 11 were used, all the males would have had nonresistant X chromosomes. All such crosses were made using females 44 because it was reasoned that this would reduce the difference between

the means of the mortality distributions of the sexes and thus reduce the error of th LD_{50}. Making the reciprocal cross would have increased the difference and the error.

RESULTS

Having obtained an LD_{50} value for every one of the 27 chromosome combinations, these data were subjected to a three-to-the-third power factorial analysis. For each chromosome pair there were three levels of genetic increment: no resistant chromosome, one resistant, and two resistant. Combining these three levels for the three pairs of chromosomes gave the 27 combinations. Early in the project it had been determined that a log-dose-probit plot of mortality data gave a better approximation to a normal distribution than any other simple transformation, and a large body of data from various crosses indicated that the genetic factors for resistance acted in a way which was additive on a logarithmic scale. Hence in carrying out the factorial analysis the logarithms of the LD_{50}'s in minutes of exposure were used.

Table 2 summarizes the results of such an analysis on the data for SyS-1002. Under Main Effects we get an answer to the question: Does the average X chromosome (or II or III) from SyS-1002 make a contribution to the resistant phenotype? The answers are unequivocal. All three chromosomes contribute something. Mean squares of these magnitudes could occur by chance alone in fewer than five experiments in ten thousand. Under First Order Interaction we discover whether, for example, a resistant X contributes equally in the presence or absence of a resistant II. Here, again, the answers are clear. There are no interactions between chromosome pairs taken two at a time. The mean squares are so low that values as large or larger should occur by chance half of the time. Finally, we find under Second Order Interactions that there is little if any interaction between the chromosome pairs taken three at a time. If any such interaction exists, it is less than four percent of the smallest of the main effects.

The breakdown of main effects into a linear and a quadratic component tells us what portion of the sum of squares for any one pair of chromosomes is attributable to a linear relationship between the mean LD_{50}'s of the three levels ($\bar{x}_0$, $\bar{x}_1$ & $\bar{x}_2$) and how much results from a quadratic component manifested by a significant deviation of $\bar{x}_1$ from the mean of $\bar{x}_0$ and $\bar{x}_2$. Here, again, the results are crystal clear. For all three chromosomes the linear component is far greater than the quadratic. For the latter, the mean squares are of an order which would be expected by chance more than a quarter of the time in the case of the X and the III and more than one tenth of the time in the case of the II. We can say, therefore, that for each pair of chromosomes, one resistant chromosome contributes half as much toward the resistant phenotype as two. Or, put in another way, there is no dominance.

In the lower portion of the table are given certain values of interest which can be deduced from the parameters we have measured. One of these is the contribution to the LD_{50} of a single chromosome of a given pair. These values differ, the

TABLE 2

Factorial analysis of the effects of chromosomes in producing resistance in SyS–1002

	Chromosome	Sum of squares	d.f.	Mean square	F ratio	P
Main effects	X	.50104	2	.25052	26.2	<.0005
	II	1.57859	2	.78929	82.7	<.0005
	III	.89363	2	.44681	46.8	<.0005
First order interaction	X–II	.01756	4	.00439	.5	>.50
	X–III	.02730	4	.00682	.7	>.50
	II–III	.02922	4	.00730	.8	>.50
Second order interaction	X–II–III	.07634	8	.00954	...	....
Breakdown of main effects						
Linear	X	.49427	1	.49427	51.8	<.0005
Quadratic	X	.00677	1	.00677	.7	>.25
Linear	II	1.56285	1	1.56285	163.8	<.0005
Quadratic	II	.01577	1	.01577	1.7	>.10
Linear	III	.88739	1	.88739	93.0	<.0005
Quadratic	III	.00624	1	.00624	.7	>.25

Chromosome		X	II	III
Mean for no resist. chr.	$\bar{x}_0$	.71279	.61213	.67841
Mean for one resist. chr.	$\bar{x}_1$	.91210	.85551	.86820
Mean for two resist. chr.	$\bar{x}_2$	1.04421	1.20145	1.12248
Grand mean	$\bar{x}$ .88970			
Contribution of one resistant chromosome to LD_{50}	$\frac{\bar{x}_2 - \bar{x}_0}{2}$	.16571±.05309	.29466±.05309	.22203±.05309
Expected LD_{50} of het. on linear scale	$\frac{\bar{x}_2 + \bar{x}_0}{2}$	.87850±.05309	.90679±.05309	.90045±.05309
Deviation of $\bar{x}_1$ from linear scale (Quadratic effect)	$\bar{x}_1 - \frac{\bar{x}_2 + \bar{x}_0}{2}$	+.03360±.09195	—.05128±.09195	—.03225±.09195

All errors show the 95 percent confidence limits: $\sigma \cdot t_{95}$

contribution of one X being least, that of one II greatest, and that of one III intermediate. The value for the X is significantly lower than that for the II at the 95 percent level. We can also compute the theoretical value of the heterozygote for each chromosome pair on the assumption that the relationship is entirely linear. By subtracting this from the corresponding $\bar{x}_1$ we obtain the deviation of the observed value from the theoretical line. In all three cases this deviation is smaller than its error. This is merely another way of showing that there is no quadratic component.

Knowing the contribution to the LD_{50} of a single chromosome of each pair, and assuming that the grand mean ($\bar{x}$) represents the value for the complete heterozygote (14–25–36), we can, by appropriate addition and subtraction, compute an expected LD_{50} for every one of the 27 combinations. In Table 3 these expected values are listed in ascending order along with the corresponding observed values and the 95 percent confidence limits of the latter. In Figure 2 these data are plotted. The regression equation of observed on expected is $y = 1.00000x \pm 0.00000$. What is more significant is that the variance of b is only 0.00243 and its standard error, 0.04930. The hypothesis that factors for resistance are additive on a logarithmic scale fits very closely with the experimental observations.

TABLE 3

Expected and observed log LD_{50}'s of combinations of chromosomes of SyS–1002 and Sy

Rank order	Combination	Expected log LD_{50}	Observed log LD_{50}	$-t\sigma$	$+t\sigma$
1	11–22–33	.20730	.31065	.23391	.35999
2	14–22–33	.37301	.35917	.27442	.41216
3	11–22–36	.42933	.43214	.32382	.45018
4	11–25–33	.50196	.58447	.53598	.62894
5	44–22–33	.53872	.61538	.57194	.66479
6	14–22–36	.59504	.62067	.57498	.66478
7	11–22–66	.65136	.70136	.65303	.74925
8	14–25–33	.66767	.58907	.53304	.64480
9	11–25–36	.72399	.65036	.57984	.71648
10	44–22–36	.76075	.71764	.67498	.76026
11	11–55–33	.79662	.77245	.69617	.83987
12	14–22–66	.81707	.85726	.81977	.89487
13	44–25–33	.83338	.81445	.74502	.89334
14	14–25–36	.88970	1.01413	.95859	1.07347
15	11–25–66	.94602	.76647	.72051	.81079
16	14–55–33	.96233	1.00609	.95103	1.06609
17	44–22–66	.98278	.89498	.79990	.98398
18	11–55–36	1.01865	.91703	.86002	.97678
19	44–25–36	1.05541	.88937	.84218	.93512
20	14–25–66	1.11173	1.08483	.98062	1.17958
21	44–55–33	1.12804	1.05402	.99476	1.10648
22	14–55–36	1.18436	1.15382	1.08545	1.21379
23	11–55–66	1.24068	1.28020	1.15484	1.38228
24	44–25–66	1.27744	1.30647	1.23896	1.37404
25	44–55–36	1.35007	1.41865	1.36182	1.47370
26	14–55–66	1.40639	1.52388	1.48615	1.55879
27	44–55–66	1.57210	1.68693	1.64410	1.73310

In Table 4 we have the results of exactly the same type of analysis on the data obtained from SyS-102. In this line also we have a highly significant main effect for each chromosome pair. There are no significant interactions between chromosome pairs taken two at a time and the second order interaction is extremely small in comparison with the main effects.

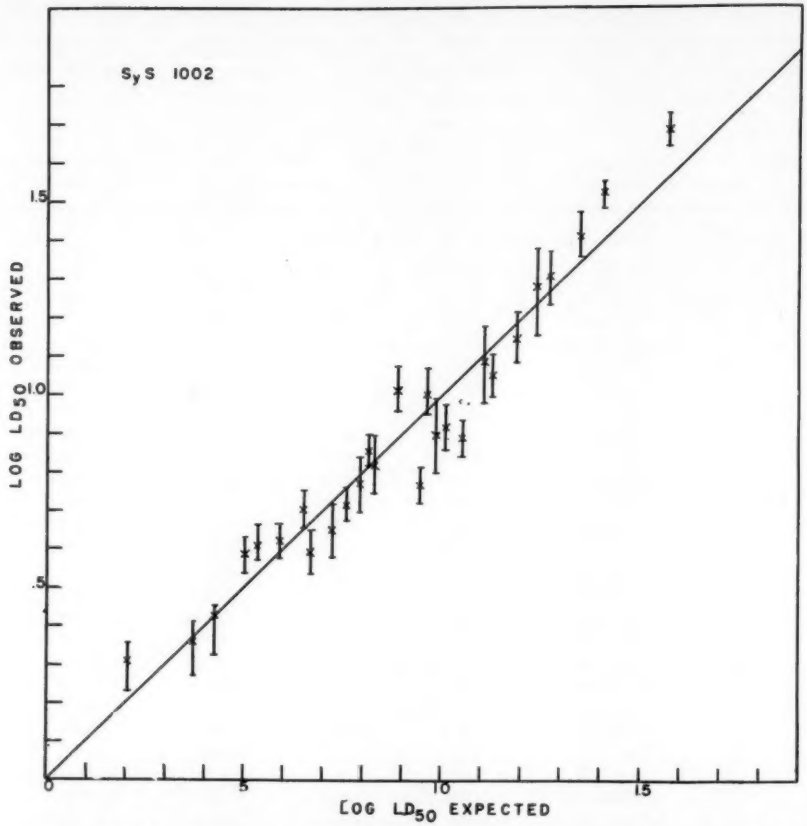

FIGURE 2.—Plot of observed log LD_{50}'s against expected values computed from contributions of individual chromosomes to LD_{50}. Crosses show LD_{50}'s; bars give 95 percent confidence intervals. Solid line is that of calculated regression equation.

Coming to the breakdown of main effects, we find one startling difference from what we found in SyS-1002. In SyS-102 there is a highly significant quadratic component for the X chromosome. There is no such component for either the II or the III. When we look at the deviation of the heterozygous X from the midpoint on the linear scale, we see again evidence of this quadratic component. This deviation is more than twice its error at the 95 percent level. The corresponding deviations for the II and III are both smaller than their errors. This means that in SyS-102 the resistant factors on the X chromosome are almost completely dominant.

The contributions of single chromosomes are unequal as they were in SyS-1002,

TABLE 4

Factorial analysis of the effects of chromosomes in producing resistance in SyS–102

	Chromosome	Sum of squares	d.f.	Mean square	F ratio	P
Main effects	X	1.95643	2	.97822	135.1	<.0005
	II	1.04275	2	.52138	72.0	<.0005
	III	.82015	2	.41008	56.6	<.0005
First order interaction	X–II	.04702	4	.01176	1.624	>.25
	X–III	.00308	4	.00077	.106	>.975
	II–III	.05101	4	.01275	1.761	>.10
Second order interaction	X–II–III	.05789	8	.00724		
Breakdown of main effects						
Linear	X	1.73012	1	1.73012	239.0	<.0005
Quadratic	X	.22512	1	.22512	31.9	<.0005
Linear	II	1.03813	1	1.03813	143.4	<.0005
Quadratic	II	.00463	1	.00463	.640	>.25
Linear	III	.79982	1	.79982	110.5	<.0005
Quadratic	III	.02031	1	.02031	2.805	>.10

Chromosome			X	II	III
Mean for no resist. chr.	$\bar{x}_0$		.71465	.83999	.85922
Mean for one resist. chr.	$\bar{x}_1$		1.21886	1.10795	1.12820
Mean for two resist. chr.	$\bar{x}_2$		1.33473	1.32030	1.28081
Grand mean	$\bar{x}$	1.08941			
Contribution of one resistant chromosome to LD_{50}	$\frac{\bar{x}_2-\bar{x}_0}{2}$		.31004±.04623	.24016±.04623	.21080±.04623
Expected LD_{50} of het. on linear scale	$\frac{\bar{x}_2+\bar{x}_0}{2}$		1.02469±.04623	1.08015±.04623	1.07002±.04623
Deviation of $\bar{x}_1$ from linear scale (Quadratic effect)	$\bar{x}_1-\frac{\bar{x}_2+\bar{x}_0}{2}$		+.19417±.08010	+.02781±.08010	+.05819±.08010

All errors show the 95 percent confidence limits: $\sigma \cdot t_{95}$

but here the X makes the largest contribution and the III the smallest. The largest and smallest contributions are significantly different at the 95 percent level.

If, using the contributions of single chromosomes, we make the appropriate additions to and subtractions from the grand mean, we obtain a set of expected LD_{50}'s for all combinations and these together with the corresponding observed values and their 95 percent confidence limits are listed in ascending order in Table 5. A regression on the two sets of variables gives the equation: $y =$

TABLE 5

Expected and observed log LD_{50}'s of combinations of chromosomes of SyS–102 and Sy

Rank order	Combination	Expected log LD_{50}	Observed log LD_{50}	$-t\sigma$	$+t\sigma$
1	11–22–33	.32841	.38651	.33313	.42567
2	11–22–36	.53921	.58844	.53034	.64548
3	11–25–33	.56857	.52692	.48077	.56557
4	14–22–33	.63845	.67033	.63574	.70752
5	11–22–66	.75001	.61088	.55467	.66097
6	11–25–36	.77937	.69223	.65148	.73336
7	11–55–33	.80873	.50897	.47212	.54112
8	14–22–36	.84925	1.00377	.95368	1.05350
9	14–25–33	.87861	.98138	.94706	1.01526
10	44–22–33	.94849	.82912	.77994	.88786
11	11–25–66	.99017	1.01895	.96975	1.06759
12	11–55–36	1.01953	1.00597	.91093	1.10133
13	14–22–66	1.06005	1.15576	1.09419	1.22639
14	14–25–36	1.08941	1.23753	1.18816	1.28398
15	14–55–33	1.11877	1.28909	1.25425	1.32085
16	44–22–36	1.15929	1.09219	1.04277	1.14037
17	44–25–33	1.18865	1.16604	1.12876	1.20250
18	11–55–66	1.23033	1.09300	1.03813	1.14909
19	14–25–66	1.30021	1.42918	1.39445	1.46141
20	14–55–36	1.32957	1.51774	1.48152	1.55146
21	44–22–66	1.37009	1.22293	1.17511	1.26979
22	44–25–36	1.39945	1.28300	1.22190	1.34014
23	44–55–33	1.42881	1.37463	1.34029	1.40853
24	14–55–66	1.54037	1.68495	1.61827	1.78137
25	44–25–66	1.61025	1.63632	1.60390	1.66958
26	44–55–36	1.63961	1.73295	1.62234	1.95704
27	44–55–66	1.85041	1.67536	1.61057	1.73063

$0.99998x + 0.00002$, with a variance of b of 0.00459 and standard error of 0.06775. This variance is 1.89 times that which we obtained in the regression for SyS-1002. As an F-ratio this is not quite significant at the 95 percent level. Inspection of Table 5 suggests that a substantial portion of this variance arises from the 14 combinations which are consistently above their expected values. This is not surprising since the factorial analysis told us that the resistant X was dominant. If we correct each expected 14 value by adding to it the difference between $\bar{x}_1$ for the X chromosome and the grand mean (0.12944), we obtain a revised set of expected values corrected for dominance. These are listed in Table 6. In figure 3 the observed values are plotted against the corrected expected values. If we compute a regression on these figures we obtain: $y = 1.01368x - 0.05863$. We now have a negative intercept because we have increased some of the expected values without changing any of the observed, but the new line is essentially parallel to that for $y = x$ and the variance and standard error of b have decreased. These are now, respectively, 0.00227 and 0.04764. The value of

b is not significantly different from 1.0 and the variance is surprisingly close to that which we obtained for b in the case of SyS-1002, 0.00243. When we make allowance for the dominance, which is confined to the X, we find that the remaining factors for resistance in SyS-102 are additive on a logarithmic scale. The fact that the variances of the two b's are so nearly equal and that the mean squares of the second order interactions of the two factorial analyses differ by a factor of only 1.3 indicates that experimental error must have been very nearly constant from one analysis to the other.

DISCUSSION

There has been a great deal of discussion of whether resistance to DDT is produced by a single factor or by a complex of factors, and published findings on this point have not all been in agreement. Part of the confusion seems to arise from the fact that two different questions are involved. One is: can we find a

TABLE 6

Expected and observed Log LD_{50}'s of combinations of chromosomes of SyS–102 and Sy. Correction made for dominance by adding .12944 to the expected value of every 14 combination

Rank order	Combination	Expected log LD_{50}	Observed log LD_{50}	$-t\sigma$	$+t\sigma$
1	11–22–33	.32841	.38651	.33313	.42567
2	11–22–36	.53921	.58844	.53034	.64548
3	11–25–33	.56857	.52692	.48077	.56557
4	11–22–66	.75001	.61088	.55467	.66097
5	14–22–33	.76789	.67033	.63574	.70752
6	11–25–36	.77937	.69223	.65148	.73336
7	11–55–33	.80873	.50897	.47212	.54112
8	44–22–33	.94849	.82912	.77994	.88786
9	14–22–36	.97869	1.00377	.95368	1.05350
10	11–25–66	.99017	1.01895	.96975	1.06759
11	14–25–33	1.00805	.98138	.94706	1.01526
12	11–55–36	1.01953	1.00597	.91093	1.10133
13	44–22–36	1.15929	1.09219	1.04277	1.14037
14	44–25–33	1.18865	1.16604	1.12876	1.20250
15	14–22–66	1.18949	1.15576	1.09419	1.22639
16	14–25–36	1.21885	1.23753	1.18816	1.28398
17	11–55–66	1.23033	1.09300	1.03813	1.14909
18	14–55–33	1.24821	1.28909	1.25425	1.32085
19	44–22–66	1.37009	1.22293	1.17511	1.26979
20	44–25–36	1.39945	1.28300	1.22190	1.34014
21	44–55–33	1.42881	1.37463	1.34029	1.40853
22	14–25–66	1.42965	1.42918	1.39445	1.46141
23	14–55–36	1.45901	1.51774	1.48152	1.55146
24	44–25–66	1.61025	1.63632	1.60390	1.66958
25	44–55–36	1.63961	1.73295	1.62234	1.95704
26	14–55–66	1.66981	1.68495	1.61827	1.78137
27	44–55–66	1.85041	1.67536	1.61057	1.73063

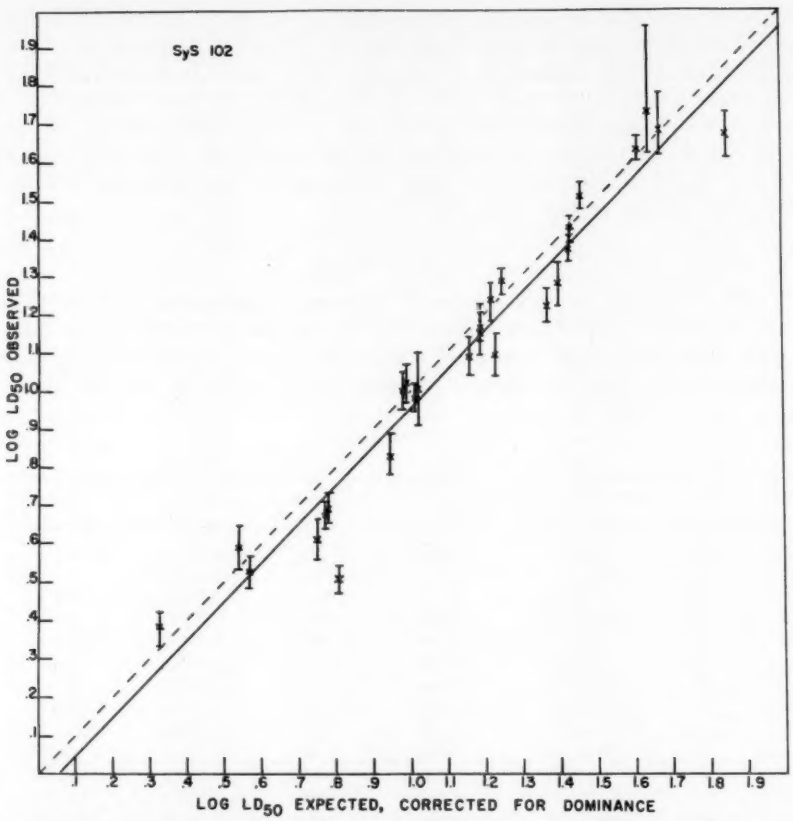

FIGURE 3.—Plot of observed LD_{50}'s against expected values computed from contributions of individual chromosomes to LD_{50} with corrections for the dominance of the X chromosome. Crosses show LD_{50}'s; bars give 95 percent confidence intervals. Solid line is that of calculated regression equation. Dotted line is that for $y = x$.

female and a male which differ in resistance and whose F_1 and F_2 offspring will be resistant or nonresistant in ratios which can be explained by regular Mendelian segregation of a single pair of alleles? The other is: are there sizable, randomly breeding populations differing in mean tolerance to DDT where this difference can be explained by a difference between the two populations in the frequencies of two alleles at a single locus?

The present analysis does not give a negative answer to the first question. We were careful to test a sample of numerous chromosomes in each of the three pairs. Our values for the contributions of individual chromosomes are therefore means for the samples of chromosomes tested. We cannot say that among the

chromosomes with which we worked a single set might not have been isolated which would have shown the major portion of resistance to segregate as a single gene. Nor can we give an unequivocal negative answer to the second question. What we can say is that we know of three populations which differ from each other with respect to several genetic factors. Each pair of chromosomes must have at least one locus affecting resistance. The X chromosome must have two such loci or three alleles at one, for it can be dominant or merely additive. There are factors which confer larval resistance without increasing adult resistance (KING 1955b). The fact that the relative potency of the three pairs of chromosomes differs between SyS-1002 and SyS-102 also argues for several factors on each. Neither of the two resistant populations tested owes its resistance to a single factor. And yet both stemmed from the same original stock and both were subjected to long and unrelenting selection. If the gene pool of the original stock had contained a gene which could produce high resistance with high efficiency, it is hard to see why it should not have become fixed as the basis for resistance in each subpopulation.

Most of the investigators who have worked on the genetics of resistance in Drosophila have concluded that they were dealing with polygenic systems (CROW 1957; BOCHNIG 1954; NACHTSHEIM and LÜERS 1954; OSHIMA 1954; SOKAL and HUNTER 1954). An outstanding exception to this consensus is afforded by OGAKI and TSUKAMOTO (1953) who found resistance attributable to a single gene which they were able to localize in a given region of chromosome II. However, the English summaries of their papers make it difficult to deduce all the details of the experimental procedures and furthermore, they were measuring larval tolerance, which seems to differ genetically from resistance in the adult.

It is in the work on resistance in pest insects, such as the house fly and the mosquito, where one finds many investigators espousing the single gene explanation. For some reason hard to comprehend many of these workers appear to take comfort and satisfaction in demonstrating that resistance is a simple Mendelian character. It is difficult to see any way in which such demonstrations contribute to the solution of problems of pest control. It is not necessary to observe simple Mendelian segregation of resistance to prove that this character is inherited. Anyone at all conversant with recent developments in the field of population genetics will not be surprised to find a complex and apparently disorderly array of graded phenotypes in any population. Such complexity and apparent disorder do not mean that the character in question is not under genetic control.

It is impossible here to review and evaluate the vast literature on the genetics of resistance in pest insects, but a brief discussion of one paper which provides very good evidence for a single gene difference can, we believe, establish that most of these papers have given an affirmative answer to only the first of the two questions posed in the first paragraph of this discussion. MAELZER and KIRK (1953) worked with a resistant Illinois strain of house flies in which about half the population survived a dose of 4μg/fly. These survivors they called "strong." Five single pair matings between strong Illinois flies and susceptible Canberra flies

gave, in two cases, all strong F_1 offspring and in three cases about half strong and half weak. It appeared that the strong Ilinois flies carried a single dominant gene and that two of the five had been homozygous and three heterozygous. The ratios in the F_2 of the two crosses involving the homozygotes were in substantial agreement with this hypothesis, but the F_2 ratios for the three crosses involving the heterozygotes deviated considerably from the expected, susceptible flies being in excess. and the three F_2 populations were statistically heterogeneous. MAELZER and KIRK suggest that this discrepancy arose because flies homozygous for the strong gene had reduced fertility. They were unable to maintain a homozygous strong stock.

The weak Illinois flies were still more resistant than the Canberra strain. Crosses between these two produced F_1's intermediate between the parents and F_2's not very different from the F_1's. The authors conclude that in the case of the weak Illinois flies "no simple genetic factor is involved whose effects are clear-cut." But in the F_2 there were a few flies (1.8 percent) which approached the Illinois strong flies in resistance.

There is no denying that these results demonstrate the presence of a single segregating factor producing high resistance. But the evidence also suggests strongly that this single factor may be a complex which under certain circumstances may remain intact through two generations, but which may also be broken up by recombination. This may be why the less resistant flies were consistently in excess in the Illinois strong × Canberra crosses and it may also explain why some F_2 flies from the Illinois weak × Canberra crosses were almost as resistant as the strong flies. In the first case the complex was broken up by recombination; in the second, something very nearly approaching it was synthesized. Surely MAELZER and KIRK's results do not give an affirmative answer to the second question and, as a matter of fact, they were careful to make no such claim.

SUMMARY

Five years of investigation of the inheritance of resistance to DDT in laboratory populations of *D. melanogaster* had led to the conclusion that resistance was produced by a polygenic complex. To confirm this, two resistant lines were subjected separately to chromosomal analysis. In the case of each line, an LD_{50} was obtained for flies of every one of the 27 possible combinations of the three large chromosome pairs: homozygous nonresistant, heterozygous and homozygous resistant for each pair. The nonresistant chromosomes were taken from the control stock from which the resistant lines had been developed by selection. The LD_{50}'s were then subjected to a factorial analysis. This showed that factors for resistance were located on each of the three chromosomes and that their apportionment among the three was not the same in the two lines. All factors were additive on a logarithmic scale except for some factors on the X chromosome in one line. The meaning of the results obtained for the problem of the inheritance of resistance in wild populations of pest insects is discussed.

ACKNOWLEDGEMENTS

We wish to acknowledge the very valuable aid and advice given us by PROFESSOR HOWARD LEVENE of the Department of Mathematical Statistics of Columbia University in helping to set up the factorial analyses. We wish also to give credit to MRS. PATRICIA D. MYERS and MRS. JUDITH K. SOBEL. The experiment owes a very great deal to their competent, intelligent, and resourceful assistance.

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COMPARISONS OF THE PREDICTED WITH ACTUAL GAINS FROM SELECTION OF PARENTS OF INBRED PROGENY OF RATS*

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DEDUCTION from genetic theory enables one to predict how much genetic gain ought to be made per generation from a known amount of selection. Knowledge of the extent of disagreement between the predicted and actual results and inquiry into possible reasons for any disagreement is of considerable importance in the field of animal breeding.

Even though there is a large body of literature on selection for various characters in animals and plants, little attempt has been made to compare the effectiveness of selection with that expected on the basis of the degree of selection and heritability.

Comparisons between the expected and observed results have been reported by DICKERSON and GRIMES (1947) for feed efficiency in swine, by LERNER and HAZEL (1947) for a production index in poultry, by KRIDER *et al.* (1946) for growth rate of swine, by FREY and HORNER (1955) on barley, and by DICKERSON *et al.* (1954) for growth rate within inbred lines of swine. These authors report no obvious discrepancy between the observed and expected in certain traits but less effectiveness than expected in others.

KYLE and CHAPMAN (1953) made a comparison of the theoretical effectiveness of selection with the actual results obtained experimentally for ovarian response to a gonadotrophic hormone in rats, using noninbred progeny only. Selection was made for high and low responses on the basis of full sister and progeny information. The average ovary weight of each litter was predicted from that of sisters of parents using the heritability for individuals derived from an earlier study by CHAPMAN (1946). This study indicated that in general the observed gains from selection for high and low responses were somewhat less effective than predicted.

The present investigation is an extension of the above study involving comparison of the predicted averages with the observed ovarian responses in inbred offspring.

MATERIALS AND METHODS

The technical details of the experiment are fully described by CHAPMAN (1946) and KYLE and CHAPMAN (1953). In the first of these studies it was found

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that 36 percent of the total variance of ovarian response was due to additive gene effects (heritability, g^2, in the narrow sense) in the random bred population which formed the foundation stock for the present selection experiment. Environmental factors common to litter mates (e^2) were found to be responsible for 22 percent of the total variance.

In the second study, on a selectively derived population, it was indicated that the earlier estimate of g^2 may have been too large and e^2 too small. This was suggested by the similarity in sizes of the correlations among full sister litter mates in the foundation stock and in the selected stock, and the lower aunt-niece correlation in the selected than in the foundation stock. When $g^2 = .22$ and $e^2 = .26$ were used in the predictions close agreement was obtained between the predicted and observed results on these same data. For this reason a heritability of .22 for ovarian weight of an individual rat was used in the present study of inbreds.

An inbred is defined here as an individual whose inbreeding coefficient (WRIGHT 1922) exceeds .0625. The comparisons of predicted with observed averages in the present study were made within inbred progeny produced by inbred parents, by "outbred" parents, and by "crossbred" parents. An "outbred" results from parents which are bred at random except for an attempt to avoid inbreeding of progeny. "Crossbred" refers to progeny from line-crossing (mating inbred, but unrelated animals), topcrossing (mating inbred males to unrelated outbred females), and bottomcrossing (mating outbred males to unrelated inbred females). The different kinds of "crossbreds" were combined for presenting results because of their similarity in degree of heterozygosity and the small number of matings available in each classification.

Since a preliminary study indicated an effect of progeny testing on gain from selection, the prediction equation should include this aspect of selection. Due to the difficulty of disentangling the progeny data used in prediction from those found in the observed results, the present analysis was confined to those litters of offspring whose parents were not progeny tested. The prediction equation for the present study was similar to that derived by KYLE and CHAPMAN (1953).[1] The predictions were made on the average ovary weights of offspring resulting from a given mating whether or not all offspring belonged to one litter.

Simple average of all offspring resulting from different matings were used as the observed values in the comparisons with the predicted ones. The test of signifi-

[1] $\bar{A}_O = f(\bar{A}_M, \bar{A}_F, n_S, n_D, n_i, r_S, r_D, f_S, f_D, A_S, A_D, g^2, e^2)$
where:
$\bar{A}_O$ = Predicted average corrected ovary weight of a particular litter of offspring.
$\bar{A}_M$, ($\bar{A}_F$) = Unweighted average of the sister average ovary weights of all males (females) in sire's (dam's) generation.
n_S, (n_D) = Total number of sire's (dam's) sisters in all litters.
n_i = Number of offspring in a particular litter.
r_S, (r_D) = Relationship between full sisters of sire (dam).
f_S, (f_D) = Inbreeding of sire (dam).
A_S, (A_D) = Average corrected ovary weight of sire's (dam's) sisters.
g^2 = Heritability of individual's corrected ovary weight.
e^2 = Fraction of variance due to environmental factors common to litter mates.

cance of differences between the observed and predicted was made by means[2] of:

$$F = \frac{\overset{N}{\underset{1}{N(\Sigma\, W\, D)^2}}}{\underset{1}{\overset{N}{(\Sigma\, W)}}\ \underset{1}{\overset{N}{(\Sigma\, W\, D^2)}}}$$ with 1 and N degrees of freedom where:

N = Number of pairs of comparisons.
W = Number of offspring on which the observed average was based.
D = Individual difference between the observed and the predicted values.

The comparisons were made by generation, on several generations combined, and over all generations within and over parental mating types. The detailed results by generation and by several generations are not presented here. The average numbers of generations of selection covering high and low response lines were 14, 12, and 6 for the parental groups of inbred, outbred, and crossbred, respectively.

RESULTS AND DISCUSSION

Inasmuch as this study was concerned with the prediction of ovarian response of inbred offspring in relation to actual response, an attempt was made, in a preliminary study, to evaluate the extent to which the character was affected by inbreeding. The average performance of inbred offspring was compared with that of their noninbred aunts, and the data were also examined for any association between the degree of inbreeding (WRIGHT 1922) and the average ovarian response. There was no indication of the trait being influenced by inbreeding.

The over-all predicted and observed results are presented in Table 1 with the averages of some of the factors involved in the predictions. The average values of sister performances, the population averages, the average "selection differentials," and the averages of numbers of sisters of the parents, and the inbreeding of the parents were weighted by the numbers of observations in each generation giving equal weight to the sisters of the sires and dams. The average of the population from which a particular parent was selected was taken to be the average of sister averages of all individuals of the same type of mating as the parent. "Selection differential" refers to the deviation of the average ovary weights of parental sisters from the population average in a given generation. Differences between the observed and predicted were expressed so that the negative sign indicates that the observed did not reach the predicted.

Inbreeding was negligible for the outbred and crossbred parents in the high and low groups whereas the average inbreeding of inbred parents was .19 for the high compared to .25 for the low response group. The average inbreeding of off-

[2] The authors are indebted to Drs. M. Kimura and N. E. Morton for derivation of this formula.

TABLE 1

Averages of the observed and predicted ovary weights of inbred offspring from parents selected for high and low ovarian response under different mating systems and the averages of some of the factors involved in the predictions

Kind of parents	Numbers of matings	Sister averages	Population averages	"Selection differentials"	Numbers of sisters	Inbreeding of parents	Relationship of parental sisters	Inbreeding of offspring	Numbers of offspring	Observed offspring avg. (A_o)	Predicted offspring avg. ($\hat{A}_o$)	Differences†
HIGH RESPONSE												
		mg	mg	mg						mg	mg	mg
Inbred	95	93.4	73.0	20.4	2.8	.29	.68	.35	2.0	76.6	80.1	—3.5
"Outbred"	46	97.6	75.5	22.1	2.6	.04	.52	.19	2.6	85.7	80.8	4.9*
"Crossbred"	16	88.4	68.3	20.1	2.7	.06	.53	.25	2.2	76.8	73.2	3.6
All	157	94.1	73.2	20.9	2.7	.19	.62	.29	2.2	79.3	79.6	—.3
LOW RESPONSE												
Inbred	111	45.6	60.5	—14.9	3.5	.37	.72	.43	2.3	55.1	54.1	—1.0
"Outbred"	36	46.0	67.0	—21.0	3.0	.01	.51	.21	2.2	61.0	61.2	.2
"Crossbred"	19	44.5	67.4	—22.9	2.4	.03	.52	.23	2.1	52.0	61.5	9.5
All	166	45.6	62.7	—17.1	3.3	.25	.65	.36	2.2	56.0	56.5	.5

* $P < .05$.
† $(A_o - \hat{A}_o)$ for high response group and $(\hat{A}_o - A_o)$ for low response group.

spring over all mating types were .29 and .36 for the high and low response groups, respectively.

The average observed ovary weights for the high response group of progeny were 76.6, 85.7, and 76.8 mg as compared to 80.1, 80.8, and 73.2 mg for the predicted from inbred, outbred, and crossbred parents, respectively. The difference of 4.9 mg for offspring from outbred parents was found to be significant at the five percent level. However, the observed average 79.3 mg over all parental mating types was not significantly different from the over-all predicted average of 79.6 mg.

For the low response lines, the observed values were 55.1, 61.0, and 52.0 mg in contrast to 54.1, 61.2, and 61.5 mg for the predicted for offspring from inbred, outbred, and crossbred parents, respectively. The averages of the observed and predicted over all mating systems were 56.0 and 56.5 mg, respectively. None of the differences between the predicted and observed for the different mating systems or over-all in the low group was found to be significant.

No obvious trend was seen in the effectiveness of selection relative to number of generations of selection, nor was there any consistent trend with increase in inbreeding of the offspring.

In spite of the fact that the predictions of offspring performance were made on the basis of varying numbers of relatives of parents and with varying degrees of inbreeding and relationship in the relatives, these complicating factors appeared to have been taken into account adequately in the equations used to give statistical effect to genetic theory.

It is noted that the agreement between the observed and expected ovary weights of inbred progeny in the present experiment appeared to be better than in the noninbred stock studied by KYLE and CHAPMAN (1953). In comparing the present study with the previous one on the noninbred progeny, it must be remembered that the heritability for individuals of .22 was used here instead of the .36 used earlier, and that the effect of progeny on the prediction was taken into consideration in this analysis and not in the earlier one.

Considering all mating systems in this study and the earlier one, the general agreement between actual observation and prediction based on estimates of additive genetic variability ($g^2 = .22$) imply that nonadditive genetic variability was not playing an important role in causing differences in this particular character. The present experiment does not, of course, provide a very precise test of the presence of nonadditive genetic variability.

The effectiveness of mass selection, at least for early generations of selection, has been abundantly demonstrated for different traits of various organisms by WINTER (1929), MACDOWELL (1915, 1917, 1920), CASTLE (1919), GOODALE (1938, 1941), MACARTHUR (1944, 1949), ROBERTSON and REEVE (1952), FALCONER (1953), LEWIS and WARWICK (1953), and others. That the mere presence of additive genetic variability does not assure the effectiveness of mass selection after many generations of selection was shown by ROBERTSON and REEVE (1952)

and RELVE and ROBERTSON (1953) in wing length in Drosophila and by DICKERSON *et al.* (1954) in several traits in swine.

The trait dealt with in this study is one for which there is no obvious adaptive value represented within the range of values observed. Likewise there was no indication from the analyses that litter size at birth or age at vaginal opening were genetically correlated with ovarian response. Following the argument of DICKERSON (1955) and others this character would not be one in which it would be expected that additive variability would have been exhausted or unavailable for selection due to previous selection pressure.

SUMMARY

Selection for high and for low response to a standard dose of gonadotrophic hormone was practiced in the rat for different numbers of generations under mating systems involving inbred progeny from inbred, outbred, and crossbred parents. Comparisons were made between the actual and predicted gains from selection. The predictions of the average ovarian responses of offspring were made from the averages of full sisters of parents based on a heritability of .22 for ovarian response of an individual.

In general, good agreement was obtained between the observed and predicted averages in both the high and low selection groups. Over all generations and mating systems of parents, the average ovary weights of the observed and expected were 79.3 and 79.6 mg, respectively, for the high selection line. The comparable values for the low group were 56.0 and 56.5 mg.

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